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CONTENTS

page

1. G. Roth:
Effect of mercury seed dressings on the health of seeds and seedlings of various crop plants and on the physiology of young plants 53

2. G. Reinken, Bonn:
Studies on the effect of the fungicide "TUZET"® on shoot growth and fruit of different apple varieties 100

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Effect of mercury seed dressings on the health of seeds and seedlings of various crop plants and on the physiology of young plants

by Georg Roth.

Introduction and formulation of problem

Seed-borne diseases of cereals constitute a whole group complexity; in unfavourable storage and germinating conditions, their causal organisms infect both seeds and seedlings (roots and haulm base in the coleoptile region). A large number of lower organisms in this group complexity are capable of greatly reducing the germinating power, seriously affecting the general health of the seedling and thus considerably lowering the yield of cereals. Various species of bacteria *(Pseudomonas)* and a number of seed-attacking fungi cause severe harm to seeds and seedlings (Roth, 1958a, b). These numerous microorganisms (bacteria, fungi) especially invade the seeds in the region of the embryo, and spread on the coleoptiles of the seedling. Wheat, rye and barley are very susceptible whereas oats are extremely resistant. The form of infection depends upon which of the causal organisms gains dominance in the shortest time. Seed-borne microorganisms include non-pathogenic and pathogenic species, the latter being sub-divided into incidence of strongly and weakly pathogenic parasites. Their negative influence is governed by the respective environmental conditions such as moisture, nature of soil, temperature etc., and thus by the manifold microbiological influences exerted by the edaphon (microbiological equilibrium). If the microbiological equilibrium is disharmonized due to reproduction of a certain causal organism, this will constitute a hazard to the seed in the nursery bed and to the young seedling. Seed is chemically treated to protect it against most causal organisms of disease that are borne by the seed.

Favourable effects — also on young seedlings — were produced as far back as the last century by carrying out treatments with various chemicals. Following the first observations of Hindorf (1888), Loew (1892), Loew and Bokorny (1889a, 1889b), research on growth stimulation reached a climax with the studies of Popoff (1931), although the hopes that yields in agriculture would undergo an increase as an outcome of these studies, were not fulfilled. A comparison of the test results published in the pertinent litera-

ture reveals that mercury seed dressings may have both favourable and unfavourable effects (Gleisberg, 1924a, 1924b, 1925; Becker, 1926; Schütz, 1931; Supper, 1932; Gassner, 1926). These varying results are to be attributed to the respective test procedures which, in the majority of cases, do not permit a comparison. Added to this are the differences in the various test factors, such as origin, age and variety of the seeds tested, varying environmental conditions during development, maturity and harvest, different soil, climatic and fertilization conditions, and differences in the treatment of the seed between harvest and sowing. Seed saprophytes and parasites which rapidly reproduce in conditions favourable for development can considerably be checked or completely eradicated by treating the seed with mercury dressings. Furthermore the secondary effect of seed treatment (Gassner, 1927) attaches great importance when the diseases are soil-borne and not seed-borne. Studies on varying temperature conditions and other seed bed factors have been published by Gassner and Rabien (1926), Müller and Molz (1925) and Molz and Müller (1925/26), in connection with the influence exerted on the germination of cereal grains and other graminaceous seeds following application of the different seed treatment methods (Krauss, 1925, 1928; Pichler, 1925; Schaffnit, 1926; Winkelmann, 1927; Pringsheim, 1928, 1928/29; Rabien, 1932; Gassner, 1943 and Rademacher, 1954).

Although we have a relatively wide knowledge of parasitic microorganisms occurring on seed and their biology, we do not have much information about seed-borne microorganisms which are harmless in normal conditions, and which vary greatly in abundance and species. From the fact that different microorganisms, especially fungi, are harmful in certain conditions e. g. in the presence of a high water content and inadequate maturity of the seed etc. (Anderson and Alcock, 1953; Armolik and Dickson, 1956; Bothomley, Christensen and Geddes, 1952; Carter and Young, 1950; Christensen, 1946, 1951, 1955a, 1955b; Christensen and Gordon, 1948; Christensen and Drescher, 1954; Christensen and Gohen, 1950; Hyde, 1950; Hyde and Galleymore, 1951; James, Wilson and Stark, 1946; Johnson, 1948; Rautenstein, 1939; Scherpe, 1899; Semeniuk, Nagel and Filman, 1947; Snow, Crichton and Wright, 1944), although appearing to be harmless in normal conditions, we must conclude that also in cases where no parasitic causal organisms can be detected, success can be achieved by treating the seed with mercury dressings. In such instances it is quite feasible that treatment of seed with mercury dressings gives the deceptive impression of bringing about stimulation or does actually produce a stimulating effect.

The problem lies in the correlations existing between the seed, the saprophytic and parasitic seed-borne microflora and the stimulating and adverse influence of seed treatment on germination and early development of the plants and the seed-borne microflora.

The influence of microflora on germination and early growth is dealt with in papers by Mannozi-Torini (1932); Schwarz-Kraepelin (1947), Isakowa (1937), Isakowa and Schmirnova (1937), Ledingham,

Sallans and Simmonds (1949), Muth (1907), Noll (1936), Ostazeski and Bewer (1955), Stille (1938), Winter (1951), Winter and von Rümker (1949, 1950) etc. It is noted that there is an alternation of stimulating and inhibiting influences on germination, and it appears obvious to assume that seed dressings have a certain selective effect on the microflora of seeds. According to Lakon (1916, 1939, 1950) and Hiltner (1887, 1903, 1911), inhibitions of germination are to be looked upon as the outcome of fungal attack which is intensified following mechanical damage caused during threshing and in the presence of embryonecroses. By means of seed treatment, reproduction of microorganisms is completely stopped or largely checked, and the seedling is given a better start.

Papers on such groups of microorganisms as bacteria, actinomycetes, fungi and yeasts associated with the seeds of our crop plants have been published by Behrens (1897), Flechsig (1954), Fowler (1924), Gentner (1914), Kent-Jones and Amos (1940), Peterson, Schlegel, Hummel, Guendet, Geddes and Christensen (1956). The first studies on the colonization of cereal grains by microorganisms were conducted by Hoffmann (1896). Several years later, a paper was published by Chrzaszcz (1902) on the microflora of barley and malt grains, in which prominence was given more to the interests of brewing. The first studies from a phytopathological aspect were carried out by Hiltner (1903, 1911, 1918). Düggeli (1904), following up on the work of Burri (1903), reported on the development of bacteria on healthy seedlings and seeds of different plant genera and plant species. His results on the incidence of *Bacterium herbicola* which, from the numerical aspect, occurs most frequently of all on healthy seed, were confirmed by Mack (1936), Mishoustin (1932), Morgenthaler (1918) and Wöller (1929). Sathe and Subrahmanyan (1931) found in their studies, two species of bacteria which, as a rule, frequently occur on seeds, but like Hofer and Hamilton (1940) they add that these microorganisms originate from the field soil in which the seed-bearing plants have grown. Recent American papers by James, Wilson and Stark (1946) and by Ledingham, Sallans and Simmonds (1949), state that *Bacterium herbicola* which mainly occurs on seeds, is a typical seed colonist; it does not adhere to the seed purely by accident but develops on it, and in fact stimulates germination and early growth. It may also protect seedlings against pathogenic bacteria and fungi living in the soil. According to Wallace and Lochhead (1951), the different seed species appear to have a specific microflora which is not connected with the flora of the respective soil where the seed plants grow. It has been established that the seed-borne microflora by no means consist of only a few bacteria but incorporate the most manifold of species which are capable of exerting very different influences on seeds and seedlings (Roth, 1958a, 1958b).

The practical importance of seed treatment undoubtedly lies in the elimination of microorganisms that are harmful to seeds. At this stage, however, reference must be made to several other views of workers conducting research on enzymes. Bach, Oparin and Wähner (1927) and Oparin and Djatschkow (1927) discovered changes in the enzyme content during the maturing of seeds. According to these studies, the catalase first increases to

a maximum value but then the catalase content drops again to the constant value for quiescent seeds (Oparin and Pospelowa, 1927). Research carried out by Bach and Oparin (1922), Oparin (1922) and Nemec and Duchon (1921a, 1921b), also suggests that enzymes are partly responsible for transformation in germinating seeds; it is furthermore concluded from these studies that there is a constant change in the enzyme activity. Results are available of more recent investigations conducted by Ruge (1947, 1948, 1952). On the grounds of the test results obtained by these research workers, it is feasible that enzymes are influenced as a result of seed treatment.

This paper has been written to provide a contribution on the specific effects of mercury seed dressings, e. g. influence on storage of seeds, and on germination and early growth of different crop plants and their seed-attendant microorganisms.

Material and methods

In order to study the influence of treatment with mercury products on the development of the seedling, the seed had to be freed of its natural microflora adhering to it. Surface sterilization of the seed is also of importance when testing the different species of bacteria and fungi for their parasitic effect on seeds and young plants. The seed was first given a thorough washing in water containing washing powder to remove the dirt sticking to the seeds. By means

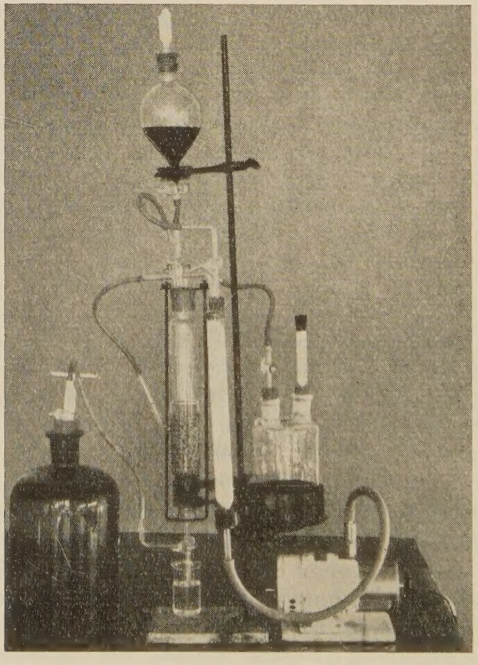

Fig. 1a

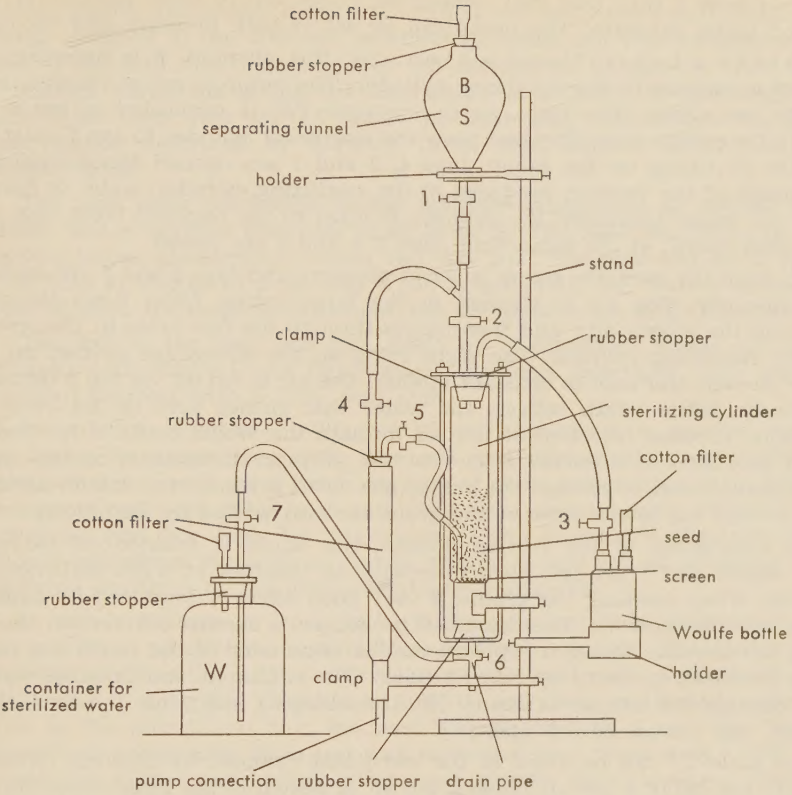

Fig. 1b

of an apparatus which can also be used for preparing suspensions of micro-organisms, it is possible to attain absolute surface sterilization accompanied by only a slight reduction of the germinating power (Figs. 1a, 1b).

Apparatus for surface sterilization of seed

The apparatus is used for preparing suspensions of bacteria and for the surface sterilization of seed; it can easily be assembled from simple equipment in any laboratory. It consists of a 6×40 centimetre glass tube (Jenaer glass) closed at both ends with rubber stoppers. A glass screen with numerous small holes on the top surface, is fitted in the bottom half. Air is delivered by an electric pump through the cotton filter into the sterilizing cylinder. The seeds are protected by cotton filters on all sides against air contamination after sterilization. The procedure is as follows:

After being washed under the water tap, the grains requiring sterilization are poured into the cylinder; the rubber stoppers are secured by means of a

clamp in such a way that they cannot be forced out of their position at the attained pump pressure. The pump can be set at both pressure and vacuum.

Intake of sterilized water: For this purpose, it is necessary to produce a vacuum in the sterilizing cylinder. The pump is set at vacuum, and all taps are closed. The large water container (W) is connected to tap 6 by means of a rubber pipe. The line from the sterilizing cylinder to tap 7 must be free. On switching on the pump, taps 4, 2 and 7 are opened simultaneously. As a result of the vacuum produced in the sterilizing cylinder, water is drawn out of the water container, the cylinder is filled to the required level, and the pump shut down; at the same time, taps 2, 4 and 7 are closed.

To wash the seed, the pump is set at pressure and taps 3 and 5 are opened simultaneously. The air is cleaned in the large cotton filter, flows through tap 5 into the screen pipe and then passes through the fine holes in the screen into the sterilizing cylinder. The seeds lying on the screen are swirled up by the air current and kept in constant motion. The air is fed off via tap 3 through the Woulfe bottle which collects the water that gushes over in the bacteria suspension process. Infection of the air through the Woulf bottle is precluded by the provision of a cotton filter. For the surface sterilization of the seed, it is recommended to change the pump over from pressure to vacuum several times so that the lower parts of the grain are also wetted by disinfectant.

On completion of the rinsing process, the liquid is drained off through tap 6; taps 5 and 3 are left open otherwise all residues of water will not be removed. When washing the grains, a very good effect is produced by adding a little washing powder. The described procedure is carried out several times, lasting ten minutes for each replication. The remainder of the sterilizing process is described in detail by Stolp (1952). The sublimate and bromine water is fed through the separating funnel (B. S.) and taps 1 and 2 into the sterilizing cylinder; tap 3 must be left open.

The material can be dried in the sterilizing cylinder by blowing through air. With the aid of a hot-air blower, the air is heated in the large cotton filter; it then flows into the sterilizing cylinder, and dries the seed in a relatively short time. The seed can be removed from the sterilizing cylinder in the inoculation box, and the tests can then be started (Roth, 1958 b).

The surface-sterilized seed was treated by the well-known dry seed dressing method, and several commercial mercury seed dressings were compared with each other in preliminary tests. In all, five approved mercury seed dressings were applied. Young plants which emerged from seed treated with CERESAN® showed the most marked differences, and therefore this product was used on all seed species in the main tests.

High quality seed of the barley variety Haisa II (produced by Messrs. Ferdinand Heine, Monsheim — 1954 harvest) was used as the test material; in 1955, this seed was sown at eight different places in West Germany. Other seeds used for comparison included oats (high quality seed of the Flämingstreue variety, produced by Lochow-Petkus GmbH, Hasselhorst — 6 origins), maize (high quality seed of the Gelber Badischer Landmais variety, produced by Südwestdeutsche Saatzucht GmbH, Rastatt, Baden — 5 origins), and beet (high quality seed of the Peragis Rot variety, produced by Kleinwanzlebener Saatzucht A. G. — 2 origins).

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The Eggebrecht (1949) method was used to test germinating power and growth vigour. Deviations from this method will be noted accordingly. As the results obtained in the different germinating media are very irregular, and in view of the fact that a germination test carried out in sand is often subject to marked variations giving the impression of a lower germinating power, most of the tests were carried out at the same time according to the tetrazolium method described by Lakon (1942, 1950, 1952) (Lindenbein and Bulat, 1955).

The tests were conducted in the Institute greenhouse at various temperatures and a relative humidity of 80 to 95 %. The seed was sown in transplanting boxes, Mitscherlich vessels and clay pots. The seed was set in soil and brick fines at depths of 2 cm and 3 cm, respectively. For testing germinating power in sand, the seeds were pressed into the moist sand (Eggebrecht, 1949).

The soil in the seed bed was made up of one-third arable soil freshly fertilized with mineral and commercial fertilizers, one-third river sand and one-third compost with a little peat dust.

In order to eliminate influences of soil microflora, a parallel test was run along the same lines; here, the soil and equipment were sterilized at 2 atmospheres gauges for 2 hours on each of three consecutive days. After the seed boxes had been filled with soil, they were covered with sterilized paper during the first few days. By this means, it was possible to eliminate the air and soil microflora for a time, that is insofar as one can at all speak of the soil being sterilized by the above-mentioned measures.

In the laboratory, a series of tests was conducted in sand, brick fines, soil and filter paper (germinating bags and petri dishes). The 18×12×2 cm sheet zinc dishes filled with sand for use in the sand tests, were sterilized for four hours in a drying chamber at 160° C, and then moistened again with water in the inoculation box; the seed was set in such a way that the seed furrow was underneath. Each test was replicated four times with 4×100 seeds. In addition to the germinating power and growth vigour, records were kept of the state of health of the plants (according to Doyer, 1938), length of the seedlings, root adherence, diameter of the coleoptile cylinder, number of roots, and microorganism attack. The seed-attendant microflora of barley seeds which have already been discussed in great detail in another publication (Roth, 1958 b), will only be referred to briefly in this paper. A comparison will be made of the test objects (barley, oats, maize, beets).

Determining the effect of seed dressings on seed of different crop plants

1. *Influence of seed treatment with mercury products on germinating power, growth vigour and seed-attendant microflora.*

   The results obtained in tests in which barley and oats were treated with several dry seed dressings did not confirm the data given in the pertinent literature that mercury seed dressings have the effect of accelerating or stimulating germination. On the contrary, in the tests carried out from November to January 1955/56 and during the summer months of 1956, it was established that germination was appreciably retarded in sand and on filter paper (in petri dishes and germinating bags) and slowed down

slightly in brick fines and in soil (Table 1). Untreated seeds germinate somewhat more quickly than treated seeds even in optimum growing conditions. However, such a difference is not only offset within a short period but moreover a completely changed situation is encountered at the end of the test. The treated seeds featured a higher germinating power and the retarded germination was made up for after a few days; in fact growth-stimulating effects of the mercury seed dressings were observed in brick fines and soil. On filter paper and in sand, the inhibiting effect on germination usually continued to persist until the end of the test especially when the germinating medium was well moistened.

Table 1: Percentages and rate of germination for treated and untreated barley.

Germinating medium and treatment	Average values after given no. of days									
	2	3	4	5	6	7	8	9	10	14
Filter paper										
untreated	47	75	83	89	92	92	93	93	93	93
CERESAN	—	—	24	53	86	90	94	94	94	94
Comparison product A	—	3	16	46	70	85	92	92	92	92
Comparison product B	—	2	29	59	82	87	91	93	93	93
Sand										
untreated	12	77	87	89	90	90	90	90	90	90
CERESAN	—	18	48	84	87	92	95	95	96	96
Comparison product A	—	14	52	70	81	90	93	93	94	94
Comparison product B	—	14	56	72	94	95	95	95	95	95
Brick fines										
untreated	—	—	—	—	—	11	52	85	87	87
CERESAN	—	—	—	—	—	7	49	86	93	93
Comparison product A	—	—	—	—	—	10	46	92	92	94
Comparison product B	—	—	—	—	—	4	51	85	94	95

The test results for oats are very similar to those for barley so that there is no need to give further consideration to them here.

Whereas maize is slightly susceptible to mercury seed dressings, the values obtained for beet seed greatly vary. It appears that the origin has considerable bearing on this. As a rule the germination results obtained for untreated beet-seed balls were better at the beginning than for those which were treated. The values are found to change, however, during the course of the test period in favour of the treated seed; the values for

untreated seed show a drop. The young plants which externally appear to be still completely healthy, may crack and wilt after a few hours; the treated plants, on the other hand, are fresh and healthy.

Table 2: Germination results for beet seed of 2 origins with greatly varying populations of microorganisms in a germinating medium with low microorganism incidence, untreated and treated (6 g of CERESAN per kilo of seed).

Treatment	Count on														
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
	12th. day			16th. day			20th. day			24th. day			40th. day		
Origin G*															
untreated	66	—	—	115	6	2	92	14	9	73	29	18	52	38	27
treated	37	—	—	73	—	—	88	—	—	107	—	—	105	—	3
Origin K**															
untreated	69	—	—	112	—	—	137	1	—	122	4	1	92	13	17
treated	29	—	—	81	—	—	83	—	—	115	—	—	116	—	—

* Origin colonized by high percentage of microorganisms.
** Origin colonized by low percentage of microorganisms.
A = No. of healthy plants per 100 beet-seed balls.
B = No. of diseased plants per 100 beet-seed balls.
C = No. of dead plants per 100 beet-seed balls.

The dying plants were laid out on different nutrient substrata in order to identify the organisms responsible for causing young beet seedlings to crack and die. The following fungi were found on diseased young plants: members of the genera *Rhizoctonia, Phoma, Fusarium, Alternaria and Cercospora beticola.* Several *Penicillium* and *Aspergillus* species, especially *Aspergillus amstelodami, Aspergillus niger* and *Phyllosticta,* occurred very frequently although not so regularly. The latter genera also occur frequently on other plants and do not appear to be specific colonists of beet-seed because they invade oats, maize and barley just as often, with the exception of *Phyllosticta* which, apart from being present on beet, was also found represented by two species on barley (Roth, 1958 a). A comparison of the laid-out seedlings originating from untreated and treated test seed very clearly shows that the latter featured a sparse incidence of microflora, usually several species of bacteria and *Penicillium.* The young plants that developed from untreated seed, on the other hand, were colonized by a large number of bacteria and fungi of the most varied genera and species. In the case of beets, the origin has great bearing especially on the seed-attendant microflora. It appears as if a negative influence on the health of the young seedlings is linked with an increasing

percentage of germinating microorganisms. The two beet origins that were examined differ as follows with respect to the average content of microorganisms: Origin G = 16 380 000; Origin K = 2 400 000.

The values given in Table 2 clearly illustrate the effect of seed treatment with mercury products. Admittedly, the success of the treatment is not evident from the first count. In fact it seems as if the germinating power might be reduced as the result of seed treatment. However, the effect of the treatment is obvious in the next counts. Whereas many of the untreated plants die as the test progresses, good results are obtained with the treated seed, and even plants that emerge late from this seed germinate and grow normally under the protection afforded them by the mercury seed dressings. The final result registered at the end of 40 days very clearly demonstrates how important seed treatment can be also in this respect.

The microflora of maize is not of the same high numerical order as that occurring on barley, oats and beets. Here, too, considerable differences were established among the various origins, especially with respect to species. The genera and species of fungus that occur most frequently on maize include *Fusarium, Gibberella zeae, Colletotrichum graminicola, Verticillium cinna-*

Fig. 2

barinum, Cephalothecium roseum and several members of *Penicillium, Aspergillus* and *Mucor*. Depending upon the origin, these fungi occur on maize seed in varying abundance. The most dangerous are undoubtedly *Gibberella zeae, Colletotrichum graminicola* and several *Fusarium* species, although *Verticillium cinnabarinum* and *Cephalothecium roseum* also produced a strong seed-deteriorating effect at high relative humidity.

Whereas *Gibberella zeae* is the main cause of seedling diseases on maize, it occurs later together with *Colletotrichum graminicola* to cause stem rot and especially spadix rot (Fig. 2). The main cause of spadix rot, however, is *Colletotrichum graminicola* (Ces.) Wils., which occurs on a large scale especially in very rainy years.

Fig. 3: Conidia of *Colletotrichum graminicola* (Ces.) Wilson

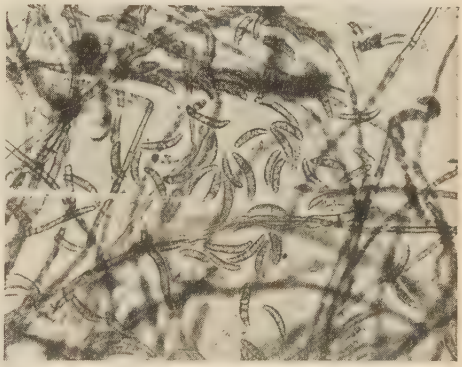


Fig. 4: Conidiophores together with conidia of *Colletotrichum graminicola* (Ces.) Wilson

Infection tests with this strain illustrated in Figs. 3 and 4 in the form of sporophores and conidiophores, were successfully carried out not only on maize but also on barley, rye and wheat. Its activity is greatly restricted by treating seed with mercury products. In fact the fungus can be completely eliminated in most cases by means of seed treatment.

Another fungus which participates in the deterioration of maize seed, and which also very frequently occurs on barley *is Verticillium cinnabarinum* (Cda). Reinke et Berth. In the living slide culture, using the method described in another publication (Roth, 1958b), upright conidiophores are formed which have sterigmata in a verticillate arrangement. The conidia are formed at the ends, which give the impression of a sporangium as shown in Figs. 5 and 6. On biomalt agar, the fungus assumes a yellowish-brown colour and its runners penetrate deep into the medium. The external pattern on the nutrient medium takes the form of irregular folds or is ribbed like a mesentery. Seed treatment with mercury products checks the spread of the fungus and lowers its vitality.

Under conditions that are favourable for its development, especially at high relative humidity, the apricot-coloured

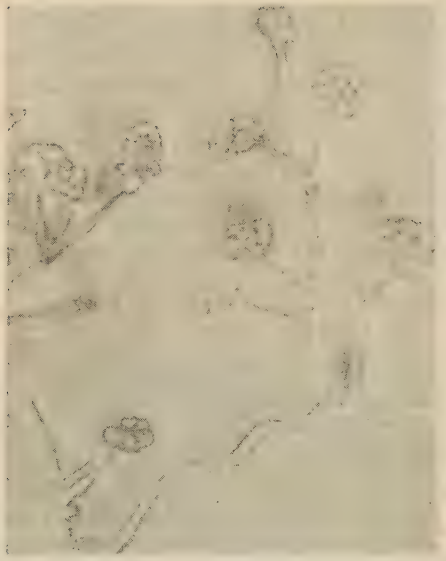


Fig. 5: *Verticillium cinnabarinum*, upright conidiophores with sterigmata in verticillate arrangement (Cda) Reinke et Berth

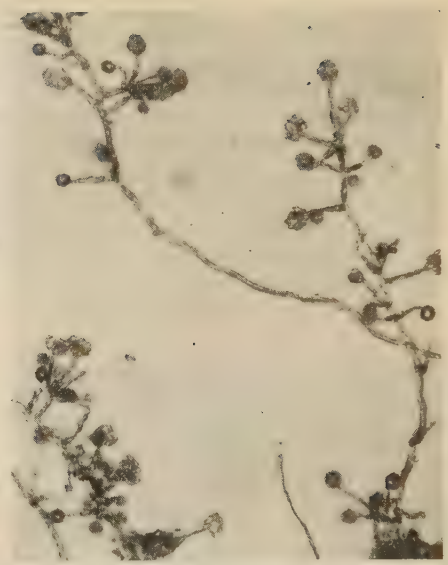


Fig. 6: *Verticillium cinnabarinum*, mycelium and conidiophores (Cda) Reinke et Berth

fungus *Cephalothecium roseum* (= *Trichothecium roseum* Link) (Fig. 7) is capable of causing really severe damage.

This fungus is also a fairly frequent attendant organism of barley seeds. Due to its rapid propagation, it is capable of very greatly reducing, within a

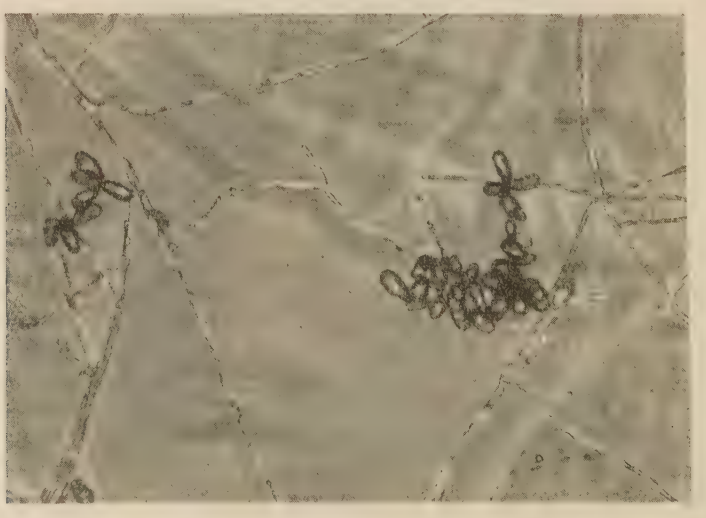


Fig. 7: *Cephalothecium roseum* with mycelium and conidiophores

short time, the germinating power of the seed, and accelerating deterioration. Its species can be easily identified microscopically because it is outstanding for its large, bicellular conidia which are elongated and pear-shaped or oval, and which form at the end of thin conidiophores. On spice agar, it forms a thick yellowish-red to pinkish-red layer within a few days. This saprophyte of seed can also be rendered harmless by means of seed treatment with mercury products.

A detailed report has already been given in another publication on the seed-attendant microflora of barley (Roth, 1958b), and therefore brief reference only will be made to it in this paper. It is evident from Table 3 that also in the case of barley, the origin has great bearing especially on resistance to many saprophytic and parasitic microorganisms. All eight origins were tested for germinating power and growth vigour in "untreated" and "treated" comparison experiments, by means of both the sand and brick fines testing method as well as the tetrazolium method of Lakon (1942). In the tetrazolium test, there was a very slight difference of approximately 1 % between "untreated" and "treated" seed with respect to both germinating power and growth vigour (tetrazolium value). On the other hand, most noteworthy values were obtained in the germinating media of sand and brick fines. Here, the dry seed dressing had a most positive effect.

In a comparative study, the origins "Gießen", "Oberer Lindenhof" and "Dikopshof" are outstanding for fungus infection. The untreated seed of these three origins, which was laid out to germinate, showed macroscopically peculiar characteristics (red and brown colour) in a large number of grains. Soon afterwards, the formation of mycelium and conidia of widely varying fungi was observed. Nevertheless, grains attacked by fungi also germinated well, and produced seedlings which appeared healthy. The fact, however, that such seedlings are severely infected and suddenly crack and die under certain conditions of fungus development (high relative humidity), will be discussed in more detail later. It is certain that already at the start of germination, the seed-attendant, harmful fungi are able to exert a decisive influence on the germinating power of the seed, but that the injuries to the young plants greatly increase when optimum conditions for fungus growth prevail. The smaller percentages of germination registered for the "Hohenheim" origin ("untreated" column) cannot be attributed to an excessively severe attack of microorganisms. The seeds that had still not germinated lay completely healthy in the germinating medium, and in fact the majority of them did germinate after the test was over.

Whereas in most cases, origins with a marked fungus infection were afflicted by a sudden overgrowth of fungus under favourable germinating conditions, it was possible to keep the same origins completely free from harmful fungus parasites by treating them beforehand with mercury seed dressings. Several *Penicillium* species showed a partiality for developing on these seeds; it has, however, already been proved that these species do not have a negative influence on germination and the development of young plants, with the exception of *Penicillium luteum* (Roth, 1958b).

Table 3 provides a comparison of test results obtained with respect to germinating power and growth vigour, also for fungus infection of "machine-threshed seed" with slight threshing injuries and fungus infection of barley seed removed from the ears by hand. The percentage of germination for seed

Table 3: Influence of dry seed treatment on germinating power and growth vigour of different origins of the Haisa II barley variety, 1955 harvest

Origin	Germinating power in sand						Tetrazolium* germinating potency		Growth vigour in brick fines				Tetrazolium* value	
	germinated		infected by fungus		still healthy						infected by fungus			
	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated
Machine-threshed seed														
Dikopshof	83	99	14	0	3	0	99	100	96	100	4	0	95	98
Gießen	82	93	18	0	0	7	98	98	93	96	7	1	95	96
Göttingen	89	98	6	0	5	2	98	99	96	98	3	0	94	95
Hohenheim	86	100	2	0	12	0	99	100	98	100	2	0	100	98
Kiel	96	98	3	0	1	2	99	99	96	98	4	0	95	97
Lindenhof	85	94	14	0	1	6	98	99	92	97	8	0	94	96
Monsheim	93	100	4	0	0	0	98	99	94	98	6	0	94	97
Weihenstephan	96	99	4	0	0	1	99	99	96	99	4	0	98	99
Seed picked by hand														
Gießen	89	95	2	0	9	5	97	99	96	98	1	0	96	97
Göttingen	99	99	0	0	1	1	100	100	97	99	1	0	99	98
Hohenheim	91	98	0	0	9	2	100	99	98	99	1	0	100	99
Kiel	92	99	3	0	5	1	98	99	95	98	1	0	98	99
Weihenstephan	86	97	0	0	14	3	100	99	99	99	1	0	99	99

* according to Lakon (1942, 1950) and Eggebrecht (1949)

damaged by threshing were somewhat slighter. It is, however, of much greater interest to establish that undamaged grains showed very slight signs of harm caused by fungi. Although it is noticed that in two cases the "Gießen" origin has a slight fungus infection, this is by no means comparable with the fungus infection of 18 % on seed damaged by threshing.

The highest percentages of germination were recorded in every case for treated grains. It was established that the highest percentage of fungus-infected seeds not treated with mercury products, was in the batch that was threshed mechanically. On the other hand, seed treatment with mercury products eliminated fungus infection on grains threshed by both methods. Studies carried out by the method of Maier (1950) have shown that slight injuries to the seed caused by mechanical threshing and the effects of these injuries on germinating power and growth vigour are responsible for rapid fungus infection. Tests carried out with seed severely injured by threshing showed that the differences in germinating power and growth vigour were more definitely in favour of seed treated with mercury dressings, although here the harmful effects of mercury are also more apparent.

Improvement of the germinating power of old seed by treatment with mercury products

All the tests that have so far been described were carried out with seed of maximum germinating power. It, therefore, appeared advisable to conduct another test series in order to establish the effect of mercury treatment on the percentage of germination for seed with a greatly weakened germinating power. For this purpose use was made of the following four barley varieties taken from the 1954 harvest:

a) Isaria barley, Wg 329; Origin: Bietenberg, Kreis Biberach; germinating power 91, growth vigour 88.

b) Barley No. 8115; origin: Kreisgenossenschaft Öhringen, germinating power 80, growth vigour 76.

c) Ochsenhausener Ria barley, high quality seed; origin: Gutsverwaltung Hohenstadt, Kreis Aalen; germinating power 71, growth vigour 68.

d) Ackermanns Donaria, Wg 42; origin: Domäne Bauhof near Sigmaringen; germinating power 66, growth vigour 63.

Tests were run at the same time with two varieties of oats which had a greatly reduced germinating power. The results are very similar to those obtained for barley, and will not be given further consideration here.

The large variations recorded in the tests on germinating power and growth vigour are very closed linked, even for a high quality seed, with the correct adjustment of the germinating conditions and the greater sensitivity and susceptilibity of embryos infected by necroses to microorganism infections (Lakon, 1916, 1939). The great bearing of the latter aspect is clearly evident from the results of the tests given in this paper. In the determination of the germinating power values, the maximum attainable percentages of germination, or at least figures closely approaching this maximum, were recorded for seed with a greatly reduced germinating power which had been treated with CERESAN. It is most definite that the more sensitive and susceptible seed is to microorganisms (especially to a number of

fungi), the more the embryos are infected by necroses. The germinating power and growth vigour of such seed can be improved by treating it with mercury products in order to eliminate most of the microorganisms that are harmful to the seedling.

Table 4: Effect of CERESAN on barley seed of low germinating power and growth vigour. Values expressed in %.

Orgin * and treatment	Germinating power** germinated	fungus-infected	still healthy	Growth vigour tetrazolium	brick fines	fungus-infected	still healthy	Tratazolium value
a) untreated	82	15	3	91	86	12	2	88
treated	87	1	12	91	92	2	6	88
b) untreated	67	33	—	80	74	26	—	76
treated	82	4	14	80	79	2	19	76
c) untreated	59	36	5	71	63	37	—	68
treated	70	12	18	71	72	4	24	68
d) untreated	52	46	2	66	58	37	5	63
treated	68	9	23	66	65	4	31	63

* see Page 67

** sand used as germinating medium

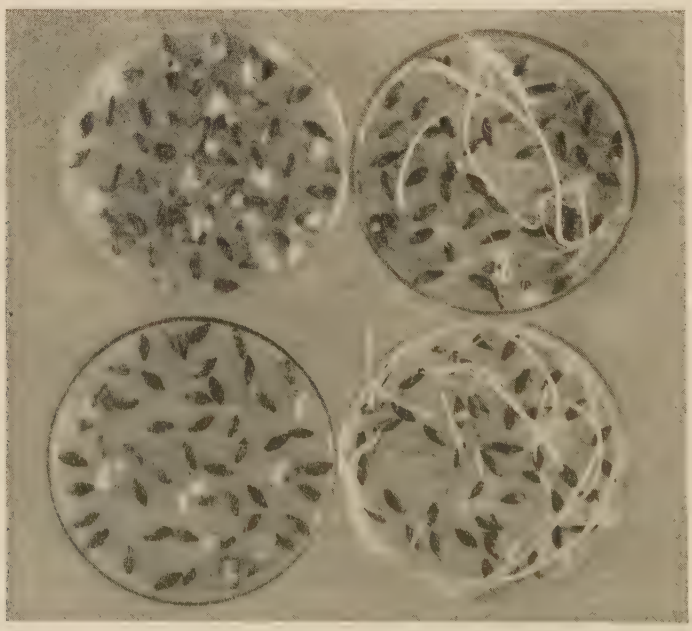


Fig. 8: Seed of low germinating power a) untreated b) treated. On filter paper in petri dishes

The results given in the column headed "fungus infected" in Table 4 show the inhibiting effect of mercury treatment on fungi. The fact that a reduction of the percentage of germination is due to the influence on the microflora becomes evident on comparing the results for untreated and treated seed in Table 4. Regardless of the germination results, the treated seed — still not germinated in the germinating medium — has a very healthy appearance, that is with very few exceptions (column headed "fungus infected" and "treated" = *Penicillium* and *Aspergillus* species), whereas the untreated seed is mostly infected and covered by the most varied of fungi (Fig. 8).

Further tests carried out on filter paper and in petri dishes produced the same positive results for treated specimens, as given in Table 4. In these tests, the untreated seeds suffered very severe damage due to rapid fungus infection; this is evident from Fig. 8a. In view of the healthy state of the young seedling, treatment with mercury products must also be regarded as having a very positive effect. Whereas microorganisms developed within a short period on untreated seed in rather moist germinating conditions, initiating deterioration of the seed, it was found that treated seed had a fresh and healthy appearance free from fungus attack, right up to the end of the test (Fig. 8b).

Germination tests with seed treated with mercury products, in moist germinating medium

All eight of the aforementioned barley origins were included in the germination tests carried out in moist conditions and at low temperature (12° C). It was assumed that optimum germinating conditions by no means always prevail when seed is sown in the fields.

The values obtained after a test period of two weeks are given in Tables 5a and 5b, and show that the percentages of germination were raised for nearly all the origins tested as the result of seed treatment with mercury products. In addition, it was established in these tests that untreated seed was severely infected by fungi followed by a marked lowering of the general health of the seedlings.

Table 5a: Germinating power expressed in % for barley (Haisa II variety), in extremely moist sand at 12° C, over 14 days. Treated with CERESAN.

Origin	Germination in percentages	
	untreated	treated
Dikopshof	18	23
Gießen	1	16
Göttingen	7	22
Hohenheim	12	24
Kiel	9	18
Lindenhof	1	23
Monsheim	4	14
Weihenstephan	13	19

The seeds that had not germinated were taken out of the seed bed after two weeks and were then sown in soil at 22° C under optimum germinating conditions. An interesting fact established was that after a further two weeks none of the untreated seed germinated with the exception of two origins (Weihenstephan with 2 % and Kiel with 1 %), whereas the percentages of germination recorded for the treated seed were most considerable (Table 5b).

Table 5b: Germinating power expressed in % for barley seed (Haisa II variety) that had not germinated in the preceding test (Table 5a), and which was now sown in soil at 22° C under optimum germinating conditions; values recorded after further 14 days.

Origin	Germination in percentages	
	untreated	treated
Dikopshof	0	51
Gießen	0	29
Göttingen	0	41
Hohenheim	0	35
Kiel	1	43
Lindenhof	0	37
Monsheim	0	36
Weihenstephan	2	46

The results given in Tables 5a and 5b show, on comparing "untreated" and "treated" seed, that treatment of seed with mercury products makes a big difference even under such extremely unfavourable germinating conditions. On making a timely change-over in the test to optimum germinating conditions as described above, it is found that treated seed will retain its germinating power, despite previous unfavourable germinating conditions, whereas untreated seed is infected by the rapidly spreading microflora.

Influence of seed treated with mercury on changes in the seed bed

Several germination tests were conducted in sand and moist soil under difficult germinating conditions to study the effect of seed treatment with mercury on the change in the degree of acidity in the germinating medium and on a shift in composition of the microorganism groups. Seed set to germinate in extremely moist conditions showed a most perceptible difference with respect to outer appearance, odour and pH value after only a few days. The fact that there is a marked difference between treated and untreated seed and the correlated seed bed pH value, is apparently based on the elimination of certain microorganisms brought by treatment with mercury products. Furthermore, the influence of such other factors as seed quality, origin and the microclimatic conditions during vegetation, maturity and harvest period should not be underestimated. An interesting feature is the positive selective effect of mercury seed dressings on a number of seed-attendant bacteria which have no adverse influence on seed germination and early growth either in pure

cultures or in mixed cultures, and which develop and reproduce well despite seed treatment. When treated seed is exposed to unfavourable germinating conditions for lengthy periods, even its germinating power is appreciably reduced in the course of time, but the break-down of organic substance is a much slower process as is also the general deterioration. In this process, a number of intermediate break-down products are formed.

When the pH-ionometer of Lautenschläger was used for carrying out the determination of acid, it was possible to register only the total acid in the form of a change in the pH value. In order to be sure of making allowance for the carbon dioxide of the air taken up by the liquid, the test included a check without seeds. It was found that the air carbon dioxide constitutes a by no means inconsiderable percentage of the acid content, but cannot be compared with the percentage of organic acids that are normally formed. Even though the percentages and types of acids that formed here were not determined, since this would have meant straying too far from the actual subject, it was nevertheless definitely established from the odour and with the aid of the comparison substances that volatile fatty acids such as acetic acid, butyric acid etc. were present. At first the pH value of the germinating medium of the untreated seed dropped below the initial value (6·9), followed by an increase in the alkaline pH range, whereas the pH value of the treated test specimes remained for a long time in the acid range. The change in the pH value varies according to the origin of the seed. It cannot, however, be said here whether these differences are to be attributed to the seed-attendant microflora of the respective origin or to other causes.

In order to obtain data on the changing microflora in the seed bed, suspensions of microorganisms were prepared by the known methods on the same day that the pH value of the germinating medium was measured. The population of microorganisms in the germinating medium, based on a uniform standard for all origins, increased very rapidly in the untreated medium (at first bacteria, and then the fungi). Seed with retarded germination was surrounded by a light layer of slime, and its germination was then really inhibited. Afterwards fungi settled on this slimy layer thus eliminating any possibility whatsoever of germination. The population of microorganisms in germinating medium containing treated seed, increased at a slow rate, and there were no fungi present, except for a few of the *Penicillium* genus, even on the 10th day of the test. The seed was perfectly healthy and had a pleasant sourish smell.

A comparison of the results for "untreated" and "treated" seed given in Table 6 shows that the tendency was the same for all the origins tested. A rapid increase in the incidence of microorganisms is followed by a marked change of the pH value in untreated medium. The seeds are exposed to big changes. Whereas a harmful incidence of microorganisms made itself apparent on treated seed only in the rarest of cases (with the exception of *Penicillium* and *Aspergillus* infection) even after two weeks had elapsed, a most active fungus flora was perceptible on the untreated seed after about only 5 days (Fig. 9); this was followed by a quick deterioration of the seed accompanied by a considerable lowering of the germinating power. The differences in the degree of acidity of the germinating medium "moist quartz sand" as registered for untreated and treated seed, suggest that the application of mercury brings about elimination of a number of harmful microorganisms.

Table 6: Changes of pH value and shifts in composition of microflora during germination tests lasting 10 days on barley of different origin, carried out in moist germinating conditions. Seed treated with CERESAN.

Origin and treatment	Change of pH value after 3 dys.	6 dys.	10 dys.	No. of microorganisms after 3 dys.	6 dys.	10 dys.
Wet sand no seed	6·8	6·6	6·6	$3{\cdot}4\times10^{2}$	$1{\cdot}5\times10^{3}$	$2{\cdot}8\times10^{2}$
Monsheim A	6·2	5·8	7·4	$5{\cdot}9\times10^{7}$	$9{\cdot}2\times10^{6}$	$2{\cdot}8\times10^{8}$
Monsheim B	6·7	6·6	6·3	$1{\cdot}3\times10^{5}$	$9{\cdot}4\times10^{5}$	$3{\cdot}2\times10^{6}$
Gießen A	6·5	6·2	7·1	$2{\cdot}7\times10^{8}$	$5{\cdot}3\times10^{8}$	$2{\cdot}5\times10^{9}$
Gießen B	6·6	6·7	6·4	$2{\cdot}9\times10^{6}$	$3{\cdot}2\times10^{6}$	$4{\cdot}7\times10^{6}$
Göttingen A	6·7	6·1	7·6	$9{\cdot}5\times10^{6}$	$7{\cdot}8\times10^{6}$	$1{\cdot}3\times10^{7}$
Göttingen B	6·8	6·3	6·4	$2{\cdot}5\times10^{5}$	$7{\cdot}1\times10^{5}$	$2{\cdot}1\times10^{6}$
Hohenheim B	6·6	5·7	6·9	$3{\cdot}9\times10^{7}$	$8{\cdot}2\times10^{7}$	$4{\cdot}6\times10^{8}$
Hohenheim B	6·8	6·7	6·7	$2{\cdot}7\times10^{6}$	$6{\cdot}8\times10^{5}$	$3{\cdot}2\times10^{6}$

A = untreated B = treated.

It can be concluded from the values obtained that when a certain microflora increases, a corresponding change of the pH values takes place. The reason is that the microorganism population of the different origins varies greatly both in number and kind, and determines the course of germination.

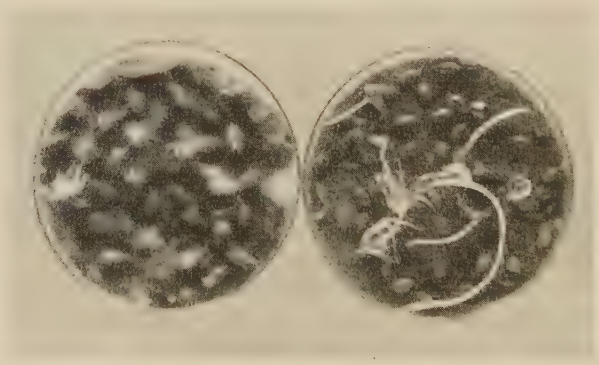
(a)  (b)

Fig. 9: Infection of microorganisms on untreated and treated seed in moist quartz sand after 5 days. a) untreated b) treated

Effects of seed treatment on the development of the young seedling

The test results tabulated and discussed in the preceding sections have shown that the development of normal, healthy seedlings very largely depends upon a reduction or elimination of the possibility of seed being infected by harmful microorganisms. The treatment of seed with mercury products constitutes such a preventive practice. It has, however, been established that treatment with mercury products not only has the effect of protecting seed

against numerous harmful microorganisms but also has a positive influence on the seedling. This effect of mercury treatment on the rate of germination and in fact on germination as a whole, has been widely discussed. In the literature, mercury seed dressings are often said to have a stimulating effect on growth (Frohberger, 1956; Nagel, 1925; Niethammer, 1929; Senf, 1925; Volk, 1927, 1930; Plaut, 1925, 1932; Stephan, 1934; Zimmermann, 1927 and others) although these findings are opposed by observations that have been to the contrary. Neuweiler (1928), Schander, Stolze and Rothmaler (1927) and other authors established no differences in the growth of young plants. Gassner (1926) often observed that growth was stimulated by seed treatment but it was not until more recently (Gassner, 1953) that this author referred to a stimulating effect being produced by wet seed dressings on lily-of-the-valley.

It can be taken as most definite that treatment of seed with mercury products stimulates the germinating power of the seed and protects it against infection. However, the fact that the positive influence of mercury treatment is not only due to the inhibition or elimination of the microflora but also to certain physiological changes in the seeds, is pointed out in papers by research workers on enzymes and by physiologists (Amlong and Naundorf, 1957; Amlong, 1938; Gracanin, 1926, 1928; Bach, Oparin and Wähner, 1927 and Ruge, 1947, 1948, 1952). Apart from those given below, no results have hitherto been published with respect to the direct influence of mercury seed dressings on seedlings and the early stages of plant growth.

In numerous comparison tests with untreated and treated barley seed, very interesting results were obtained on the influence of mercury treatment on the formation (and the number) of roots of young seedlings. The observations and results constitute average values obtained for 2 000 test plants (Table 7).

The results in Table 7 show that the different mercury seed dressings which were applied had a varying influence on root formation. Three of the products brought about an increase in the number of roots whereas the treatment with a fourth seed dressing (comparison product III) had no positive influence. The average values for this latter product were in fact below those calculated for the untreated check. The effect on the seed-attendant microflora and thus on the general health of the plants was equated with the other mercury seed dressings.

Table 7: Influence of different mercury seed dressings on the number of roots produced by barley seedlings (Haisa II variety, origin Monsheim).

Germinating medium	untreated	CERESAN	Comparison product I	II	III
Brick fines	6·24	6·85	6·77	6·68	5·78
Soil	6·18	6·45	6·38	6·29	5·95

Usually the number of roots produced in brick fines is larger than in soil. The mercury is apparently bound more firmly in soil than in brick fines, but nevertheless the favourable influence on root formation is unmistakable. As a rule, barley seedlings have six roots, and do not so often have 5 or 7. Due to

the application of mercury seed dressings, the number of roots is raised on the average to 7, in isolated cases to 6 or 8 to 10. It is clearly evident from the observations made and from the results given in Table 7, that treatment of seed with mercury products has the effect of increasing the number of roots that are formed, both in brick fines and in soil.

The young plants sampled for counting the roots, were carefully washed in water to remove soil and brick fines, respectively. An interesting point observed was that the roots of treated plants held the brick fines more firmly than those of the untreated checks. Fig. 10 illustrates plants that grew from untreated seed, with 6 roots (lack property of holding brick fines), and Fig. 11 shows plants that developed from treated seed, with 7 roots featuring good adhesion for brick fines.

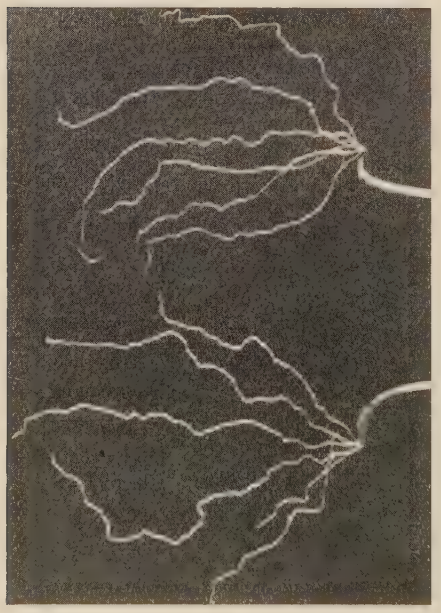


Fig. 10: Roots of young seedlings developed from untreated seed, with poor adhesive property for brick fines

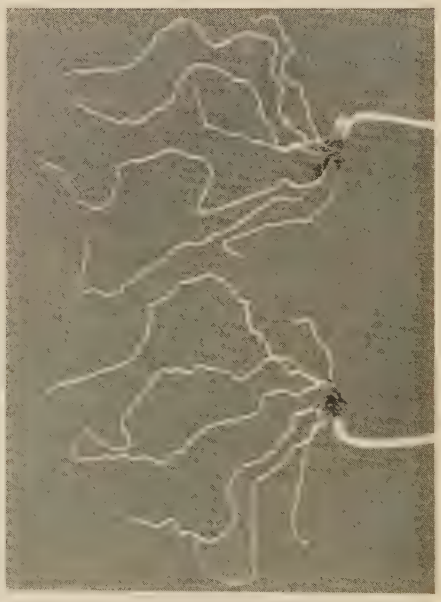


Fig. 11: Roots of young seedlings developed from treated seeds, with good adhesive property for brick fines

Fig. 12 illustrates barley plants produced from untreated seed (a) on the left, and from treated seed (b) on the right, rinsed under the water tap. Here, too, the good adhesive property of the roots on plants that have grown from treated seed (b) is clearly perceptible. The brick fines stick so firmly to the roots of these plants that they have to be rubbed with the fingers to remove the brick fines from the region of the seed. Furthermore, it is most noticeable in this illustration that the treated plants are much healthier. The only explanation for the better adhesive property of the roots of the treated test plants

Fig. 12: Plants rinsed under the water tap to test the adhesive power of the roots; a) untreated b) treated

is that due to the more pronounced windings of the roots the brick fines are tightly clasped and held better by the roots.

Influence of mercury treatment on the size of seedlings

In the tests carried out with barley, it was found that germination is retarded by mercury treatment in all germinating media. During the first few days growth is inhibited, although this condition is offset as the plants develop, and the situation is reversed after 10 to 14 days. Usually, however, the differences in length are so nonuniform even in the individual test groups that one cannot speak of the longitudinal growth of young barley plants being stimulated or inhibited. In the soil, differences in length are practically nonexistent; the scope of this paper does not permit exceptions to be discussed. Germination is retarded most in filter paper.

The test results obtained on the influence of mercury treatment on the development and turgescence of the coleoptile cylinder are of much greater interest. These studies have revealed that mercury seed dressings have certain stimulating effects on young plants. In order to determine differences in the

Table 8: Diameter of coleoptile cylinder (mean) — average based on measurements for batches of 200 ten days old barley plants; expressed in μ.

Germinating medium	Measuring point I		Measuring point II	
	untreated	treated	untreated	treated
Brick fines	995	1160	1940	2370
Soil	1310	1460	2020	2390
Soil (steamed)	1320	1555	2100	2420

sizes of treated and untreated plants, the dry weight was purposely not taken as a basis. A microscopic measuring method was selected; the plants were measured at two points on the 10th. day of growth, and the values were converted to μ. Table 8 gives the average values of the measurements for batches of 200 plants.

Mercury seed dressings obviously have a stimulating effect on the development and growth of the coleoptile cylinder. The measurements produced values for treated seedlings, which were appreciably higher than those recorded for untreated seed. Furthermore the young plants that emerged from treated seed appeared far sturdier and healthier than those which grew from untreated seed; in fact many parts of the tissue were observed to be yellow and diseased on these latter untreated plants. The differences in the state of health between

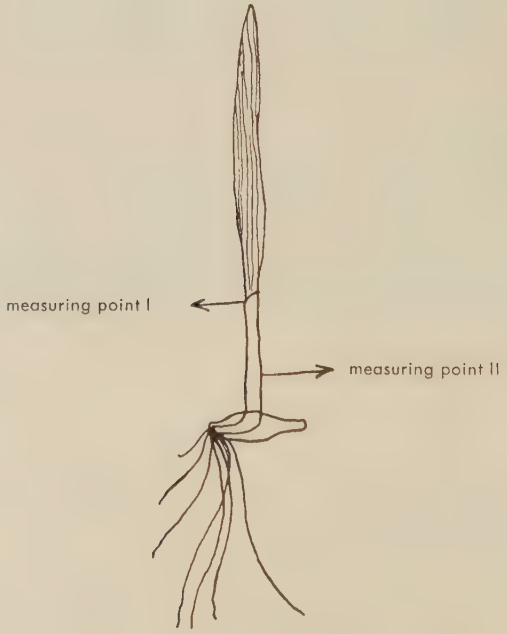


Fig. 13: Barley seedling

untreated and treated plants were especially noticeable in brick fines. Apart from their excellent condition of health, the treated plants were outstanding for their tight, turgescent coleoptile cylinders and marked freshness, whereas the untreated plants appeared dull and delicate.

Influence of mercury treatment on regeneration power of young barley plants

Two barley origins with varying microorganism population and varying germinating power (Haisa II variety, Gießen origin and Göttingen origin) were used for these tests. Ordinary soil and dampened soil were used as germinating medium. The seeds were set to germinate, and after 4 weeks the plants were cut off level with the soil surface, as shown in Fig. 14. They were then moistened and left to stand for a further two weeks. A light layer of fungus mycelium was already present on the surface of the soil at the time the untreated plants were cut.

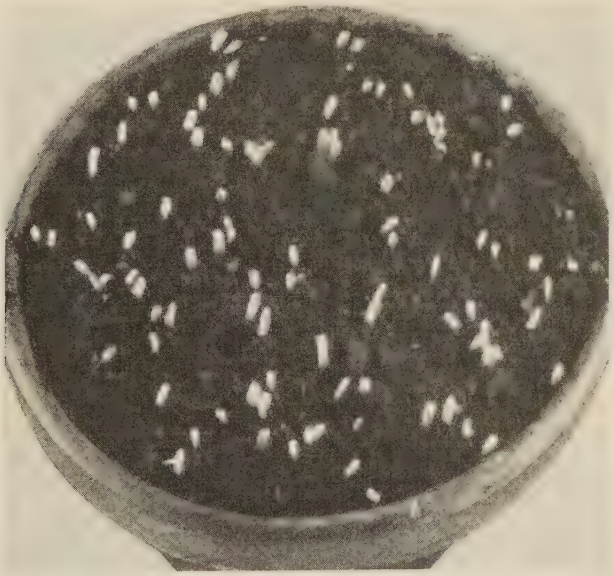


Fig. 14: Cut barley plants, four weeks old and treated; tested for regeneration power

The treated plants showed good signs of regenerating after only 3 to 4 days had elapsed whereas marked retardation was noted for the untreated plants. By the end of the test, the treated plants had recovered very well but the untreated checks were very backward in their growth (Figs. 15 to 20). The treated young plants regenerated almost completely; the number of untreated plants decreased most appreciably, especially in cases where discoloured,

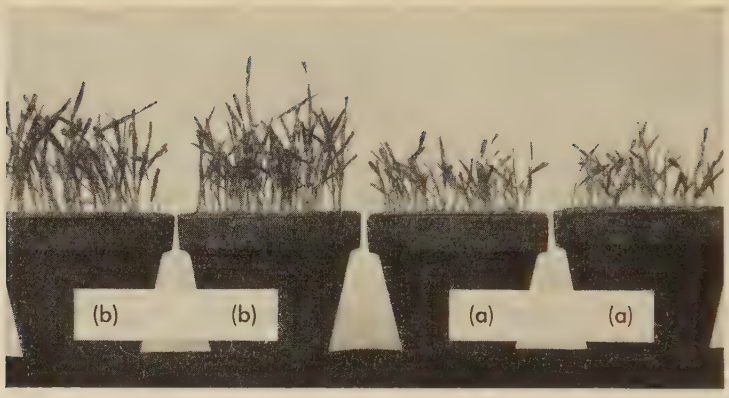


Fig. 15: Regeneration of young barley plants in 14 days; a) untreated b) treated. Soil not dampened.

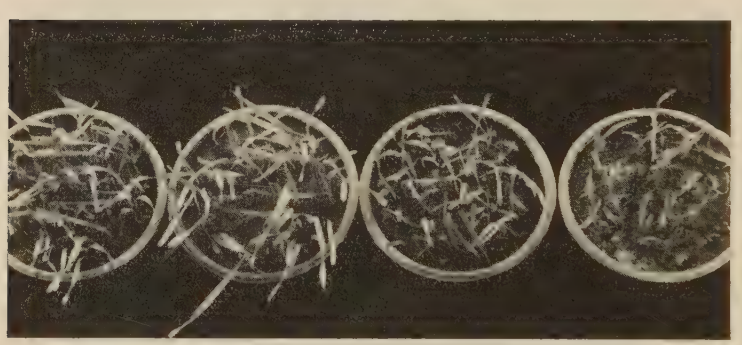


Fig. 16: As for Fig. 15. Top view.

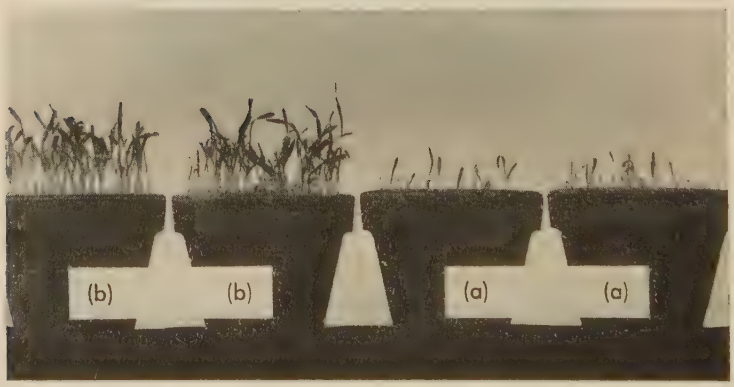


Fig. 17: Regeneration of young barley plants in 14 days; a) untreated b) treated. Soil dampened. Seed stored at 90% relative humidity.

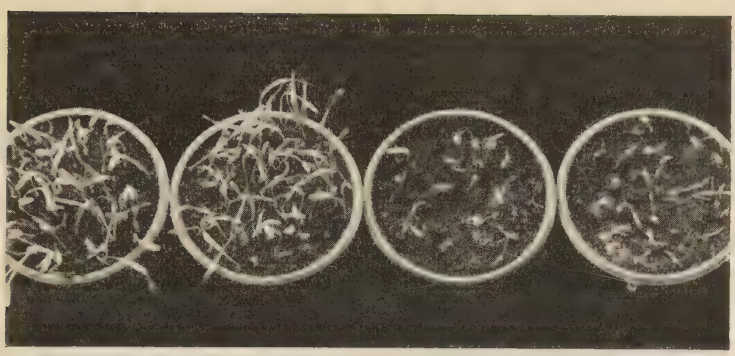


Fig. 18: As for Fig. 17. Top view.

Fig. 19: Regeneration of young barley plants in 14 days. Left to right: dampened soil, treated, untreated; soil not dampened, treated, untreated. Stored at high relative humidity.

Fig. 20: As for Fig. 19. Top view.

infected parts had been observed on the coleoptile before the plants were cut back.

Similar results were obtained for both origins (Gießen and Göttingen), as will be seen from Table 9. Even for a seed stored under normal conditions, the regeneration power of both origins drops when untreated (compare ratios of untreated and treated for origin Gießen at 81:93 and for origin Göttingen at 87:92). The results obtained for seed stored at high relative humidity (85 to 100 %) are even more outstanding. Here, the ratio of untreated to treated seed is 12:73 for origin Gießen and 17:73 for origin Göttingen in ordinary soil, while in dampened soil it is 9:79 for origin Gießen and 14:79 for origin Göttingen. These figures indicate that poor storage of seed may have an influence on the continued development of the young seedlings in the seed bed, that is not to be under-estimated. The greater regneration power of the treated plants is clearly obvious from the results in Table 9. Moreover, there is no spread of fungus on the treated plants, this being evident from Figs. 16, 18 and 20. The protective effect of mercury treatment is especially noticeable in dampened soil where the fungus very quickly spreads over the untreated cut plants. A number of injurious fungi attack the cut plants on the cut surface, and form mycelium on the plant stumps, which spreads over the entire surface of the soil in the test boxes (pots), breaking the already weakened resistance of the plants. As a result, it becomes very difficult for such plants to regenerate, and in most cases they are unable to do so at all.

Table 9: Regeneration power of 4 weeks old barley plants in soil. Origins: Gießen and Göttingen (Haisa II variety). Data recorded after 6 weeks of storage at different relative humidities; expressed in percentages.

Origin	Relative humidity during storage in %	Germinating power				Regeneration percentages after 14 days			
		Soil		Dampened soil		Soil		Dampened soil	
		untreated	treated	untreated	treated	untreated	treated	untreated	treated
Gießen	check*	95	94	92	89	91	93	73	84
	75	93	95	90	86	82	92	73	84
	80	86	90	89	84	74	89	64	82
	85	72	90	80	85	56	83	48	84
	90	68	86	65	81	42	80	41	79
	95	59	85	54	79	24	78	18	76
	100	53	81	57	82	12	73	9	79
Göttingen	check*	96	93	89	90	87	92	78	87
	75	96	94	91	87	84	94	83	85
	80	92	91	88	89	77	90	81	83
	85	84	89	82	89	62	86	59	87
	90	76	87	73	86	35	83	39	82
	95	61	84	59	83	29	81	23	78
	100	51	86	54	79	17	73	14	79

*Storage in normal conditions in the loft

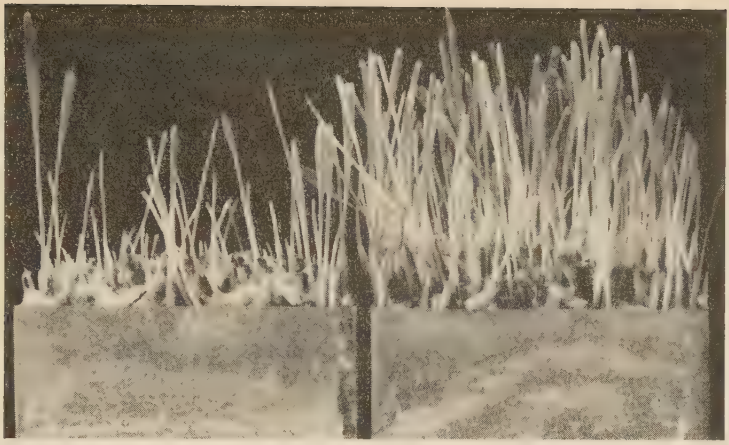


Fig. 21a: Infection tests with *Gibberella zeae* (Schw.) Petch. Regeneration power of young barley plants emerged from untreated seed (left) and treated seed (right)

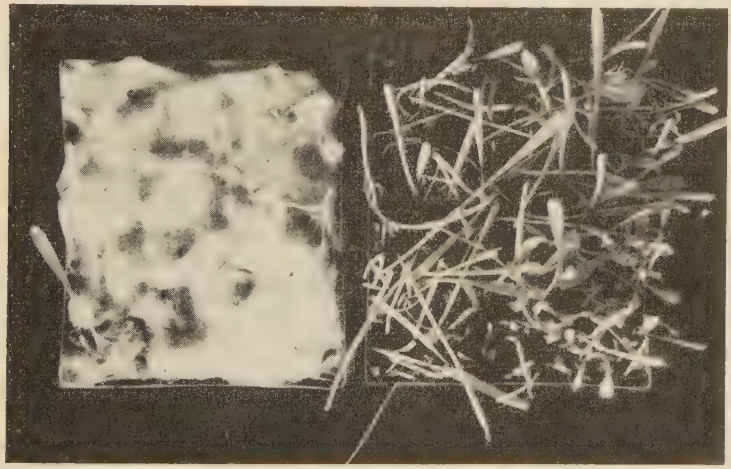


Fig. 21b: As for Fig. 21a. Top view

The fungus that occurred most frequently on the untreated seed was *Gibberella zeae* (Schw.) Petch. It causes severe damage to seed and young plants of barley, rye, wheat, oats and maize. The brown, infected parts present on gramineae are usually the result of attack by this fungus. This seedling parasite was found to be the cause of infection on 98 out of 100 diseased plants which were examined. Only very few of the plants attacked by this fungus regenerate, as shown in Figs 16, 18, 20, 21 a and 21 b. At first, seed treatment with mercury products is very effective against this fungus but when the

plants are frequently moistened the mercury seed dressing is washed out, and the fungus reoccurs after a certain time, especially in infection tests. However, the fungus is not so active as on untreated seed because on treated plants several *Penicillium* species develop in the region of the roots, which have a certain antibiotic effect on *Gibberella zeae*. Furthermore, this fungus is very susceptible to secretions of certain species of bacteria (*Pseudomonas* species) (Roth, 1959*). Therefore, plants that have emerged from treated seed develop much better, feature excellent turgescence and are very healthy; furthermore, they regenerate very well and also remain healthy after they have regenerated.

Finally, it may be concluded that plants produced from untreated seed are generally less resistant, less turgescent and not so healthy as treated plants. When they have severe injuries, untreated plants are very quickly attacked by saprophytic and parasitic microorganisms; their growth is checked or they may be completely destroyed even when produced from high quality seed. As mercury seed dressings produce specific effects on certain physiological processes taking place in the plant, apart from their preventive and protective effect against injurious microorganisms, application of these products is strongly recommended in all cases.

Influence of mercury seed treatment on germinating power and seed-attendant microflora in moist storage conditions

It is an old-established fact that when seed is stored in unfavourable conditions, e.g. in places where the relative humidity is too high, the germinating power of this seed is reduced. Such deterioration of seed brought about by microorganisms undoubtedly attaches economic importance. If such seed were to be sown, the yield would most certainly be correspondingly lower. In order to obtain data on the protective effect which mercury seed dressings afford seed against the harmful microflora, tests were conducted with barley, oats, maize and beets. The seed was stored according to the method described by Roth (1956b), in Neubauer dishes containing 350 g of barley seed (Gießen and Göttingen origins), 300 g of oat seed (Gießen origin), 350 g of maize seed (Kenzingen origin) and 150 g of beet seed (Einbeck origin), respectively. Attention was given primarily to the germinating power of the seed during and after storage, and to the microorganisms responsible for causing deterioration of the seed.

The tests were carried out at room temperature (20 to 22° C). The relative humidity was raised from 75 % in stages of 5 % to levels of 80 %, 85 %, 90 %, 95 % and 100 %. The remainder of the seed was stored in the loft, and served as a check for the tests.

The seed stored at high relative humidity absorbed considerable quantities of water in the course of time. Samples of both barley origins exposed to 100 % relative humidity were taken after 6 weeks. The water content for the Gießen

* This paper is to be published later but as it is not yet known in which periodical it is to appear, it is not included in the references.

origin was 23·5 %, and for the Göttingen origin 21·9 %. The water content values at other degrees of relative humidity are given in Table 10.

The other seed species reacted in a similar manner. At 100 % relative humidity, oats and beet had a somewhat higher water content than barley, whereas the water content of maize was slightly lower.

Table 10: Influence of increased relative humidity on the water content of barley seed of different origins.

Relative humidity in %	Gießen origin		Göttingen origin	
	untreated	treated	untreated	treated
check	9·4	9·7	8·4	8·4
75	9·8	9·9	9·3	9·5
80	10·2	10·3	10·7	10·5
85	13·5	13·1	12·9	13·4
90	16·9	17·0	16·7	17·2
95	19·2	19·5	18·9	19·4
100	23·5	23·2	21·5	21·9

With respect to preservation of germinating power and growth vigour, the values for the checks and those for seed stored at low relative humidity were within the limits of error. At high relative humidity, however, the percentages of germination for untreated seed dropped considerably after 6 weeks, and a vigorous development of fungus was observed on the seeds set to germinate in sand and brick fines (Tables 11 to 14). There was a marked shift in the composition of the microflora in favour of fungi at high relative humidity (85 % to 100 %). These changes in the microbiological equilibrium on the different seed species are very clearly reflected in the decreasing percentages of germination.

A comparison of the test results for untreated and treated seed clearly illustrates the favourable effect produced by mercury seed dressings in raising the percentages of germination and also in suppressing the injurious microflora, especially the harmful fungi. Even more striking are the findings after a storage of six months (cf Table 11). Studies conducted by Carter and Young (1950), Schneider (1954), Snow, Chrichton and Wright (1944), Tuite (1955, 1957) and Walter (1950) confirm that high degrees of relative humidity (85 to 100 %) are essential for optimum mycelium development and spore germination of saprophytic and parasitic fungi.

The microflora of the seed changes in a most characteristic manner as the water content of the seed rises and the relative humidity increases. During the first few days and weeks there is a marked increase in the bacterial flora at 90 to 100 % relative humidity, followed by a relative decrease in bacteria and an increase in fungi (Roth, 1958 b). Similar conditions were established also on oats, maize and beets.

Table 11: Germinating power and growth vigour of untreated and treated barley (Haisa II variety. Gießen origin) after storage at increasing degrees of relative humidity.

Relative humidity %	Germinating power in sand						Tetrazolium germinating potency		Growth vigour in brick fines				Tetrazolium value	
	germinated		fungus infected		still healthy				fungus infected					
	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated
After 6 weeks' storage														
check	86	92	5	2	9	6	98	96	94	95	6	5	97	94
75	88	90	8	1	4	9	95	95	92	92	8	0	92	92
80	89	91	3	2	8	7	93	95	89	91	11	2	91	90
85	65	90	17	3	13	7	85	90	77	82	23	5	78	89
90	56	68	36	8	8	24	80	88	74	82	26	11	76	85
95	44	59	48	26	7	15	74	85	48	77	52	12	52	78
100	36	60	62	19	2	21	71	82	45	75	55	14	47	74
After 6 months' storage														
check	84	90	9	2	7	8	—	—	93	94	7	6	—	—
75	86	89	10	2	4	9		—	91	94	5	2	—	—
80	77	82	9	3	14	15		—	87	92	8	2	—	—
85	58	74	39	18	3	8			62	79	38	9		—
90	6	39	94	58	0	3	—	—	8	39	92	61	—	—
95	0	0	100	100	0	0	—	—	0	4	100	96	—	—
100	0	0	100	100	0	0	—	—	0	0	100	100	—	—

Table 12: Germinating power and growth vigour of untreated and treated oats (Flämingstreue variety, Gießen origin) after storage at increasing degrees of relative humidity; 1955 harvest

Relative humidity %	Germinating power in sand						Tetrazolium germinating potency		Growth vigour in brick fines				Tetrazolium value	
	germinated		fungus infected		still healthy						fungus infected			
	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated
After 6 weeks' storage														
check	76	89	12	4	12	7	94	92	91	89	7	2	90	91
85	45	84	31	4	24	12	87	92	75	90	22	1	84	89
90	58	72	35	10	7	18	82	89	78	88	17	3	79	84
95	51	68	49	8	0	24	65	76	67	73	26	6	63	82
100	48	73	52	10	0	17	69	78	68	72	29	16	61	74

Table 13: Germinating power of untreated and treated maize (Gelber Badischer Landmais variety) after 6 weeks' storage at increasing degrees of relative humidity; 1955 harvest (Kenzingen origin)

Relative humidity %	Germinating power				Tetrazolium	
	Sand		Soil			
	untreated	treated	untreated	treated	untreated	treated
check	92	91	89	90	95	94
85	84	94	68	95	92	94
90	82	92	88	90	89	94
95	52	68	45	78	79	86
100	54	76	53	75	75	87

Table 14: Germinating power of untreated and treated beet seed (Peragis Rot variety, Einbeck origin) after 6 weeks' storage at increasing degrees of relative humidity; 1955 harvest.

Relative humidity in %	Germinating capacity in sand								Tetrazolium test			
	No. of germinations from 100 seed-balls in 7 days		14 days		No. of seedlings produced from 100 seed-balls in 7 days		14 days		No. of germinable seedballs 100		No. of germinable seeds produced from 100 seed-balls	
	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated
check	95	96	97	97	181	207	197	223	93	94	198	227
85	87	96	88	97	162	213	167	218	91	98	197	234
90	94	94	94	94	204	205	208	210	96	92	197	205
95	89	92	92	93	159	201	165	205	94	93	173	197
100	93	88	95	89	189	186	195	188	98	90	205	166

Whereas a vigorous development of fungus was observed after only a few days on untreated seed stored at such high relative humidity, treated seed was in storage for some considerable time before it was attacked by fungi of the *Penicillium* and *Aspergillus* genera. In fact a favourable condition for the development of several species of these two genera is that the seed has been treated with mercury products. The reason for the numbers of fungus being nevertheless high on treated seed at high relative humidity is that *Penicillium* and *Aspergillus*, in particular, sporulate strongly under such conditions whereas the fungi occurring on untreated seed feature strong development of mycelium (Figs. 22, 23).

From this, it is clearly evident that the fungi usually colonize the seed in the region of the embryo at the beginning, and then spread over the seed.

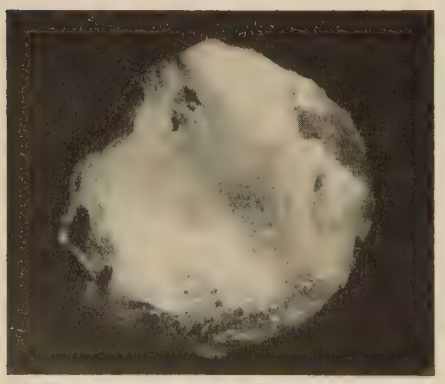


Fig. 22: Untreated maize seed attacked by various fungus species

Fig. 23: Treated maize seed (attacked by *Aspergillu* and *Penicillium*)
Stored for 6 months at high relative humidity

The *Penicillium* and *Aspergillus* species occurring on treated seed apparently do not affect germination so much as the genera of fungi occurring as parasites on untreated seed; this is confirmed by the germination results in Tables 11 to 14. Whereas the untreated seeds were embraced by a thick fungus mycelium after six months of storage at high relative humidity (Figs. 22, 24 to 27 c), and almost or completely lost their germinating power depending

Fig. 24: Barley

Fig. 25: Oats

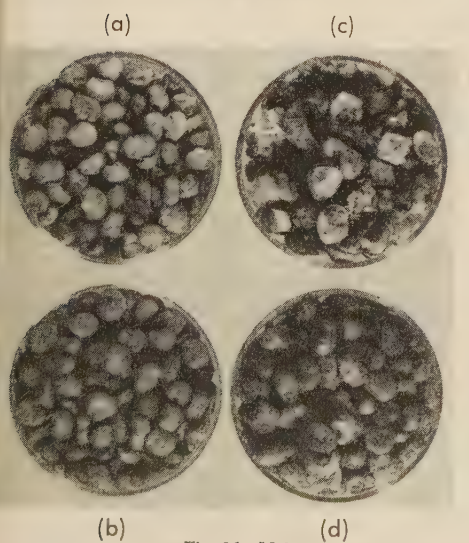


Fig. 26: Maize

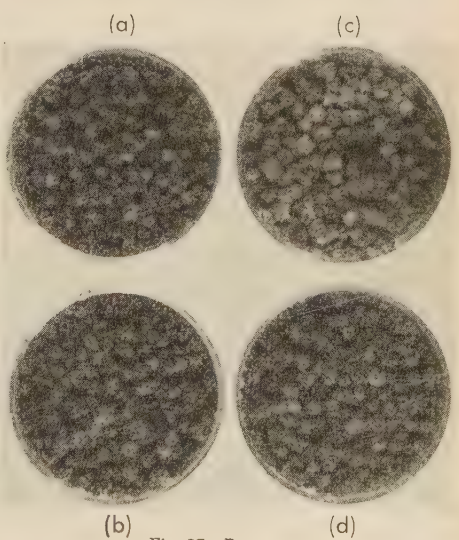


Fig. 27; Beet

Before storage a) untreated b) treated
After 6 months' storage at high relative humidity c) untreated d) treated

upon the degree of moisture prevailing during the period of storage, the treated seed of all four species tested featured higher values of germinating power and less fungal infection despite unfavourable storage conditions. The deterioration of the seed set in much later and proceeded at a slower rate than for untreated seed.

In microbiological studies of barley seed, the numerical incidence of bacteria, actinomycetes and fungi on the different origins, and the occurrence of special physiological groups of bacteria and fungi were determined, also the influence of the latter on germination and growth of seedlings (Roth, 1958 b). In this section, brief data will be given on the numerical incidence of microorganisms on oats and maize after storage periods of 6 weeks and 6 months. Attention has not been given to a determination of the species but it might be pointed out that most of the saprophytes and parasites occurring on these seeds were the same as those already closely studied for barley. There are a few exceptions, for example an abundance of *Colletotrichum graminicola* on maize, and *Alternaria, Phoma betae, Rhizoctonia solani* and *Pythium* on beet. Usually there is a strong incidence of widely varying actinomycetes on oats, but the remainder of the microflora on this plant is the same as that on barley.

Table 15: Bacterial population on untreated and treated oats (Flämingstreue variety, Gießen origin), after storage at high relative humidity.

Relative humidity in %	Initial germ content		Germ content after 6 weeks		6 months	
	untreated	treated	untreated	treated	untreated	treated
Check	$2{\cdot}9\times10^6$	$1{\cdot}8\times10^6$	$3{\cdot}0\times10^6$	$1{\cdot}4\times10^6$	$2{\cdot}5\times10^6$	$0{\cdot}9\times10^6$
85	—	—	$3{\cdot}2\times10^6$	$1{\cdot}3\times10^6$	$4{\cdot}7\times10^6$	$1{\cdot}6\times10^6$
95	—	—	$1{\cdot}2\times10^7$	$1{\cdot}7\times10^6$	$2{\cdot}9\times10^7$	$1{\cdot}5\times10^6$
100	—	—	$6{\cdot}4\times10^7$	$4{\cdot}5\times10^6$	$5{\cdot}9\times10^7$	$2{\cdot}8\times10^6$
Actinomycetes						
Check	$6{\cdot}9\times10^4$	$5{\cdot}2\times10^4$	$6{\cdot}8\times10^4$	$6{\cdot}2\times10^4$	$5{\cdot}9\times10^4$	$6{\cdot}3\times10^4$
85	—	—	$7{\cdot}0\times10^4$	$5{\cdot}6\times10^4$	$6{\cdot}4\times10^4$	$6{\cdot}2\times10^4$
95	—	—	$8{\cdot}9\times10^5$	$2{\cdot}7\times10^5$	$1{\cdot}2\times10^6$	$6{\cdot}4\times10^4$
100	—	—	$2{\cdot}9\times10^6$	$9{\cdot}8\times10^5$	$7{\cdot}4\times10^6$	$6{\cdot}9\times10^5$
Fungi and yeasts						
Check	$2{\cdot}7\times10^3$	$5{\cdot}2\times10^1$	$4{\cdot}6\times10^3$	$1{\cdot}2\times10^1$	$2{\cdot}8\times10^3$	$2{\cdot}9\times10^1$
85	—	—	$6{\cdot}3\times10^3$	$2{\cdot}5\times10^2$	$9{\cdot}1\times10^4$	$8{\cdot}7\times10^2$
95	—	—	$5{\cdot}8\times10^5$	$7{\cdot}5\times10^3$	$1{\cdot}9\times10^6$	$3{\cdot}2\times10^4$
100	—	—	$1{\cdot}3\times10^6$	$8{\cdot}9\times10^3$	$2{\cdot}4\times10^6$	$3{\cdot}2\times10^5$

Table 16: Microorganism population on untreated and treated maize (Gelber Badischer Landmais variety, Kenzingen origin), after storage at high relative humidity.

Relative humidity in %	Initial germ content untreated	Initial germ content treated	Germ content after 6 weeks untreated	Germ content after 6 weeks treated	Germ content after 6 months untreated	Germ content after 6 months treated
Bacteria						
Check	$4{\cdot}9\times10^{5}$	$9{\cdot}2\times10^{3}$	$3{\cdot}7\times10^{5}$	$4{\cdot}1\times10^{4}$	$4{\cdot}2\times10^{5}$	$4{\cdot}2\times10^{4}$
85	—	—	$3{\cdot}5\times10^{5}$	$6{\cdot}2\times10^{4}$	$6{\cdot}9\times10^{5}$	$5{\cdot}7\times10^{4}$
95	—	—	$9{\cdot}3\times10^{5}$	$7{\cdot}4\times10^{4}$	$1{\cdot}7\times10^{6}$	$2{\cdot}3\times10^{5}$
100	—	—	$1{\cdot}2\times10^{6}$	$4{\cdot}2\times10^{5}$	$3{\cdot}2\times10^{6}$	$6{\cdot}8\times10^{5}$
Actinomycetes						
Check	$7{\cdot}4\times10^{3}$	$7{\cdot}5\times10^{3}$	$6{\cdot}8\times10^{3}$	$5{\cdot}4\times10^{3}$	$2{\cdot}4\times10^{2}$	$5{\cdot}9\times10^{3}$
85	—	—	$7{\cdot}4\times10^{3}$	$6{\cdot}4\times10^{3}$	$6{\cdot}7\times10^{3}$	$4{\cdot}2\times10^{3}$
95	—	—	$1{\cdot}2\times10^{4}$	$7{\cdot}8\times10^{3}$	$9{\cdot}9\times10^{3}$	$5{\cdot}6\times10^{3}$
100	—	—	$3{\cdot}5\times10^{4}$	$1{\cdot}8\times10^{4}$	$4{\cdot}9\times10^{5}$	$4{\cdot}7\times10^{4}$
Fungi and yeasts						
Check	$9{\cdot}1\times10^{2}$	$3{\cdot}4\times10^{1}$	$7{\cdot}5\times10^{2}$	$1{\cdot}7\times10^{1}$	$1{\cdot}2\times10^{3}$	$2{\cdot}3\times10^{1}$
85	—	—	$7{\cdot}4\times10^{3}$	$3{\cdot}8\times10^{1}$	$8{\cdot}4\times10^{3}$	$6{\cdot}9\times10^{2}$
85	—	—	$9{\cdot}2\times10^{4}$	$5{\cdot}8\times10^{3}$	$5{\cdot}4\times10^{5}$	$9{\cdot}8\times10^{3}$
85	—	—	$8{\cdot}4\times10^{5}$	$6{\cdot}2\times10^{3}$	$1{\cdot}8\times10^{6}$	$6{\cdot}7\times10^{5}$

On studying Tables 15 and 16, it is noticeable that the number of fungi increases considerably as the relative humidity rises and the period of storage lengthens. The numerical values for the treated seed, on the other hand, are again much lower. When seed infected by microorganisms is used for sowing, large gaps appear especially with untreated seed (cf Tables 11 to 14). The more unfavourable the germinating conditions and the longer the seedling takes to pierce through the surface of the soil, the bigger will be the gaps. Close microscopic studies will reveal that in the region of the seed and on the fine roots, fungi and bacteria develop vigorously; these attack diseased parts on the embryo and from here they spread the infection over the whole of the seed. The findings speak for themselves. The more necrotic parts there are on the embryos, the more sensitive and susceptible

these will be to many microorganisms, and the more mercury treatment will contribute towards raising the percentages of germination by eliminating seed-pathogenic organisms. A report has already been given in another paper on the influence which seed treatment with mercury products has on the different microorganisms also in pure cultures (Roth 1958 b).

Summary of Results

The object of this paper has been to study the influence of seed treatment with mercury products on seed-deteriorating microflora in natural and difficult germinating conditions and also on certain secondary effects in the early growth stage of the seedling. It has been established that the mercury seed dressing has the effect of inhibiting germination and early growth during the first few days in the seed bed, but that after a further few days this situation is reversed. The treated plants grow vigorously after about 2 weeks, and have a healthy, fresh appearance, whereas untreated plants are less resistant to injurious microorganisms. The secondary effects of mercury seed treatment are especially as follows: development of a sturdy, turgescent coleoptile cylinder, production of a larger number of roots, increased root growth and a better regeneration power of the young plants. Furthermore, it was established that mercury seed treatment had a favourable effect on germination percentages and growth vigour especially for seed of poor origin, low germinating power and which had a high incidence of microorganisms and was stored in poor conditions. The roots of plants that emerged from treated seed were found to have a stronger adhesive power for holding brick fines than roots of plants from untreated seed.

Germination and growth vigour tests were conducted with 8 barley origins, 6 oat origins, 5 maize origins and 2 beet origins. Very marked differences were established. The influence of the origin is determined by the seed-attendant microflora which reduces the values for germinating power to a degree dependent upon the incidence of injurious species. Practically all fungi had a negative influence on seeds and seedlings. By treating the seed with mercury products, it was possible to render harmless or destroy most of the saprophytic and parasitic fungi that attacked the seeds. On the other hand, fungi of the *Aspergillus (Aspergillus amstelodami)* and *Penicillium (Penicillium luteum* group) genera are resistant to mercury seed dressings. In fact treatment of seed with mercury products is a condition for the vigorous development of these particular fungi. Most fungi begin their work of destruction on seeds in the region of the embryo, and then spread over the whole of the seed. The most severe fungal infection and the biggest decrease of germinating power were observed on seed contaminated with extensive necrosis on the embryo. Such embryos can be given a good start for their development by treating the seed with mercury products, thus eliminating many harmful microorganisms.

A number of fungi of widely varying genera were isolated and identified on barley, oats, maize and beets. Furthermore the bacterial, actinomycete and fungal flora was determined for a maize and an oat origin after storage at increasing degrees of relative humidity. These tests very clearly showed that many seed-attendant fungi and several bacteria play a role in the deterioration of the seed. It is necessary for seed to be treated with mercury products even in cases when it is completely free of fusarium, smuts and causal organisms of stripe because there are many potentially harmful organisms on the seed which are able to attack it in the seed bed through the fine injuries caused by threshing, to retard the process of germination and finally to destroy the seedling. It was also established that seed treatment with mercury products brings about a change of the pH value in the seed bed.

Fungi responsible for the deterioration of seed are as follows:

On barley: *Gibberella zeae* (Schw.), Petch., *Aspergillus amstelodami* (Mangin) Thom et Church, *Aspergillus candidum* Link, *Aspergillus repens* (Cda), de Bary, *Botrytis cinerea* Pers., *Cladosporium herbarum* Link, *Epicoccum nigrum* Link, *Fusarium avenaceum* (Mr.) Sacc., *Fusarium equiseti* (Cda) Sacc., *Fusarium oxysporum* Schl., *Mucor varians* Pispek, *Penicillium* (members of several groups: *Pfefferianus, Notatum, Elegans Chrysogenum, Luteum,* and *Purpurigenum* group), *Phyllosticta spec.; Stemphylium consortiale* (Thüm.) Groves et Skolko, *Syncephalastrum racemosum* (Cohn) Schreit., *Trichothecium roseum* Link and *Verticillium cinnabarinum* (Cda.) Reinke et Berth. The most severe harm to seeds and seedlings, however, is caused by *Gibberella zeae.*

On oats: The microflora on oats is very similar in its composition of species to the microflora occurring on barley. *Gibberella,* however, was observed only on one origin. On the other hand, a number of lower fungi were discovered which were not identified. There was no incidence of fungi belonging to the genera *Phyllosticta* and *Syncephalastrum racemosum.* A great variety of actinomycetes were found on oats. The number of species was considerably higher than on barley.

On maize: Most of the fungus species already discovered on barley were also found on maize. Fungi that occurred the most frequently on maize stored at high relative humidity, and which are also responsible for the deterioration of the seed, include: *Fusarium* species, *Gibberella zeae, Colletotrichum graminicola, Verticillium alboatrum, Verticillium cinnabarinum, Cephalothecium roseum, Penicillium luteum* and *Aspergillus amstelodami.* Whereas *Gibberella zeae* and *Colletotrichum graminicola* occur the most frequently on untreated maize seed, and play the biggest role in the deterioration of the seed, they are found to be lacking on treated maize seed (storage at high relative humidity). The fungi that cause deterioration of treated seed include members of the *Penicillium luteum* group and *Aspergillus amstelodami.*

The following fungi were discovered as being associated with seed of the two beet origins which were studied: members of the genera *Rhizoctonia, Phoma, Fusarium, Alternaria, Cercospora beticola, Penicillium* (several species), *Aspergillus niger, Aspergillus amstelodami* and *Phyllosticta.* Furthermore, three phycomycetes were isolated but the species were not identified.

These studies have established that it is absolutely necessary to treat seed, this being especially important in cases where seed has to germinate in unfavourable conditions.

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Studies on the effect of the fungicide "TUZET®" on shoot growth and fruit of different apple varieties

by G. Reinken, Bonn

In order to meet present-day market requirements, orchardists are now making increasing efforts to produce deep-coloured fruit, especially in regions where climatic conditions do not tend to enhance the colour of fruit. For this reason, there is a growing tendency to plant red-fruit varieties and to select parent stock, tree forms, pruning methods and soil cultivation practices which have a positive influence on fruit colour. When planning and preparing the indispensable crop protection and pest control measures, progressive growers choose products which not only feature high efficacy, but also — especially in the case of fungicides — have positive "secondary effects" on fruit growth and leaf formation.

Studies conducted by Hoffman (1933), Heinicke (1938), Brody and Childers (1938), Christopher, Pieniazek and Jennings (1944), Mitchell and Childers (1944), Picket, Fish and Kwong Shue Shan (1951) and Johnsen, Kennworthy and Mitchell (1950) have revealed that crop protection products influence photosynthesis and leaf growth, especially the thickness of the palisade parenchyma, on fruit trees. According to Palmiter and Smock (1955) and Palmiter and Hamilton (1955), an effect is exerted on the formation of leaf pigment, and on the size, colour and ripening of the fruits. In this respect, lime-sulphur has been found to have an adverse effect. Luckwill (1957) established that spray applications of lime-sulphur impair the formation of flower buds, and increase flower shedding and June fall. In recent years, Toorenaar (1956) and Guilliams and Soenen (1955) have reported that TUZET has a favourable influence on leaf formation, fruit colour and yield of Cox's Orange, as compared with other fungicides. Rijsten (1956) and Roosje and Huissen (1957) also mention an improvement of colour especially on red-fruit apple varieties, combined with earlier ripening.

Toorenaar (1956) and Roosje (1957) point out, however, that following the application of this product, inhibition of shoot growth, premature autumn colouring of foliage and damage to fruit peel may be caused on several varieties.

The effect of TUZET (combined product containing TMTD, ziram and an arsenical active substance — dimethyl dithiocarbamate of methyl arsine) to enhance the colour of fruit, which had been observed by orchardists, prompted us to carry out studies in 1956 and 1957 on the effects of staggered spray applications on the yield, size, colour and keeping quality of different apple varieties. As there was the possibility of shoot growth being influenced due to the 4·5 % arsenic content of the product, and considering that such a negative effect had apparently been established on certain varieties in isolated orchards, we also included measurements of shoots in the tests. The fungicidal effect of the product was not given consideration.

® = Registered trade mark of Farbenfabriken Bayer Aktiengesellschaft, Leverkusen.

Test conditions and method

The tests were carried out at the plantation of the Institute for Fruit Growing, located about 2 kilometres west of Meckenheim and approximately 180 metres above sea level.

Rainfall in 1956 amounted to 764 mm (186 rainy days), and in 1957 to 634 mm (158 rainy days). Between April 1 and September 30 there was 506 mm of rainfall in 1956 and 337 mm during the same period in 1957. Seeman and Caspar (1957) carried out a detailed study of climatic conditions in this area.

The soil consists of loess well saturated in bases with a depth of 18 metres, and weathered to brown earth down to a depth of 60 centimetres; the top soil is made up of 40·2 % washable constituents, 2·2 % humus and has a water capacity of 54 %. The plants have no supply of ground water. The pH value increases from 6·7 to 7·0 in the layers ranging from 0 to 60 centimetres. There is a good supply of potash, phosphoric acid and magnesium in these layers, especially in the main root zone at a depth of 20 to 40 centimetres.

The trees were planted in 1953 in a 3×1·5 metre hedge formation running from north to south. The James Grieve, Geheimrat Oldenburg, Cox's Orange, Golden Delicious and Jonathan varieties had an M IX parent stock. All the trees were trained on an existing frame of four wires with their lateral shoots growing horizontally, at spacings of 50 centimetres; they were pruned in the usual way every winter. The long shoots not forming part of the frame, and which naturally grow upwards, were brought into a horizontal position by binding them.

In 1956 the soil was tilled until the middle of August to keep it loose. In 1957, tillage was continued until the middle of July when white clover was then sown.

In 1955, before the trees were planted, the soil was treated with 1000 kilos of potash sulphate per hectare, 1200 kilos of Thomas phosphate per hectare and 22 000 kilos of stable manure per hectare; as a result of this treatment and on the grounds of a soil examination which showed that the soil was adequately provided with nutritive substance, a dosage of pure nitrogen in the form of ammonium sulphate was first applied in Spring, 1957 at a rate of 60 to 180 kilos per hectare depending upon the growth of the different varieties. This ensured good shoot growth and also vigorous development of healthy foliage.

The crop protection measures were carried out according to the warnings and recommendations issued by the Orchardists and Vegetable Growers Advisory Organization, Bonn. The schedule of TUZET spray applications is given in Table 1, according to which one to three sprays were carried out yearly. Four plots each containing at least twenty trees were laid out for each variety, including a check plot, and plots which were treated with TUZET on the first date, on the first and second dates and finally on all three dates, respectively. It was only on the early maturing James Grieve and Geheimrat Oldenburg varieties that three treatments were not carried out because the third spray application would have been too close to the harvest date (TUZET = Poison Class 1). In 1956 Dithane was used as the comparison

Table 1: Date and time of TUZET sprays, and prevailing weather during the applications.

Date of application	Time	TUZET conc. in %	Comparison fungicide		Insecticide		Weather conditions		
			Product	Conc. in %	Product	Conc. in %	Temp. in %	Sky	Rainfall in mm
25. 7. 56	15.00	0·1	Dithane	0·2	Basudin R	0·12	22·2	cloudy	0·0
19. 8. 56	20.00	0·075	Dithane	0·2	—	—	18·9	sunny	0·0
10. 9. 56	20.00	0·05	Dithane	0·2	—	—	19·8	bright	0·5
2. 7. 57	10.00	0·1	Orthozid 50	0·25	Basudin R	0·12	20·0	cloudy	0·6
19. 7. 57	14.00	0·055	Orthozid 50	0·25	Basudin R	0·12	16·0	cloudy	0·0
27. 8. 57	20.00	0·05	Orthozid 50	0·25	—	—	11·8	overeast	1·2

fungicide, and in 1957 Orthocid 50. The sprays were applied on the dates and at the concentrations specified by the manufacturers*. At the time of the treatment, the fruits were at least walnut-size and had little hair covering.

It is evident from the data in Table 1 that in both years the treatments were chiefly carried out in the afternoon and evening.

In 1956, only elongation shoot growth was measured on the centre and lateral shoots, whereas in 1957 the entire increased growth of the annual wood and the number of shoots were determined. The yield for each tree was determined by counting and weighing the fruit. Until it was sorted, the fruit was stored in fresh air in 1956, whereas in the second test year it was kept in a mechanically cooled store (with air washing) at + 4° C.

Sorting of the fruit in sizes was carried out according to the strictest of principles. 40 fruits of each of the sizes 70/75, 75/80 and 80 85 mm, from each variety and plot, were placed in a mechanically cooled store (with air washing) at + 4° C and 95 % relative humidity, and evaluated every four weeks until the end of storage. The colour evaluation of these fruits was based on standard fruits of the different varieties with gradings 1 to 8, and carried out by three persons in each case. Fruits with the weakest covering colour were placed in grade 1, and the most deeply coloured fruits in grade 8.

Test results

The measurements of new shoot growth showed that the TUZET treatment had no influence on the early maturing varieties James Grieve and Gehcimrat Oldenburg, either in 1956 or in 1957; this is confirmed by Table 2. The differences in shoot growth in the various plots are slight. In the case of the James

* The recommendation of Farbenfabriken Bayer A G reads as follows: "TUZET" is applied at a concentration of 0·1 %. The strength of the spray solution can be reduced to 0·075 % during the course of the vegetation period". (The editors).

Grieve variety, the length of the individual shoots increased in the first test year as a result of the TUZET treatment, and decreased in the second year.

In 1956 it was found that the entire new shoot growth was stimulated on Cox's Orange without any increase in the length of the individual shoots, but this was not confirmed in 1957.

In contrast to this, the reaction especially of Golden Delicious to every TUZET treatment in both test years was for the new shoot growth to be reduced, although the analysis of variation does not show this to be significant. The length of the individual shoots was slightly influenced in both years but a definite tendency was not established. On the other hand, it was observed in 1957 that the number of shoots, i. e. burst buds was reduced.

The reaction of the Jonathan variety in the first year to an increasing number of TUZET spray applications was also for shoot growth to be inhibited proportionally, although there was no marked repetition of this effect in the following year. The length of the individual shoots did not change appreciably in either test year; it was only in 1957 that the TUZET treatment caused a slight decrease in the number of shoots.

Table 2: New shoot growth on different apple varieties following varying TUZET treatment in 1956 and 1957 at Klein-Altendorf.

Variety	TUZET treatment	New shoot growth in cm		Growth output figure		Length of individual shoot		No. of shoots 1957
		1956	1957	1956	1957	1956	1957	
James Grieve	untreated	195·5	297·6	100	100	48·9	44·2	6·4
	one spray	176·0	310·7	90·0	104·4	51·9	40·9	7·0
	two sprays	203·6	303·7	104·1	106·0	52·9	39·8	7·5
Geheimrat Oldenburg	untreated	140·3	330·8	100	100	43·8	51·1	6·7
	one spray	140·2	313·3	99·9	94·7	39·5	45·2	7·0
	two sprays	158·5	333·3	113·0	100·8	43·2	48·8	6·5
Cox's Orange	untreated	180·6	320·7	100	100	50·5	47·0	7·1
	one spray	196·8	341·1	109·0	106·4	49·6	42·4	8·1
	two sprays	199·9	317·8	110·7	99·1	49·8	40·9	7·6
	three sprays	205·0	320·8	113·5	100	52·1	44·4	7·2
Golden Delicious	untreated	198·6	392·8	100	100	49·7	32·2	12·6
	one spray	185·4	340·0	93·3	86·6	48·4	28·8	11·7
	two sprays	182·2	301·9	91·7	76·9	49·9	29·5	10·4
	three sprays	177·4	342·8	89·3	87·3	47·6	32·2	10·6
Jonathan	untreated	233·0	497·3	100	100	58·3	42·1	12·0
	one spray	229·5	482·0	98·5	96·9	61·6	45·7	11·1
	two sprays	222·2	656·0	95·4	131·9	58·5	47·1	10·1
	three sprays	215·9	488·3	92·7	98·2	58·3	42·9	11·1

Table 3: Influence of varying TUZET treatment on yield, number of fruits and average individual fruit weight of different apple varieties in 1956 and 1957.

Variety	TUZET treatment	Yield in kg		Yield in %		No. of fruits		Average individual fruit weight	
		1956	1957	1956	1957	1956	1957	1956	1957
James Grieve	untreated	1·85	5·09	100	100	14·8	35·4	125·0	143·7
	one spray	2·29	4·99	123·8	98·0	16·9	35·9	130·0	139·1
	two sprays	2·94	5·56	158·9	109·2	20·9	37·2	140·7	149·4
Geheimrat Oldenburg	untreated	4·49	0·70	100	100	45·4	7·9	98·9	88·6
	one spray	4·32	1·70	96·2	242·9	45·0	10·0	96·1	161·9
	two sprays	4·73	0·80	105·3	114·3	51·6	7·4	91·8	108·1
Cox's Orange	untreated	1·81	4·56	100	100	15·5	30·7	116·9	148·8
	one spray	1·80	5·05	99·4	110·7	13·7	36·3	131·7	138·9
	two sprays	1·81	5·40	100·0	118·4	16·4	45·9	110·3	117·6
	three sprays	1·70	5·13	93·9	110·5	14·6	33·7	116·5	152·3
Golden Delicious	untreated	7·41	13·43	100	100	53·6	94·2	138·2	142·5
	one spray	6·57	12·82	88·7	95·5	47·4	84·3	138·6	152·2
	two sprays	5·57	10·46	75·2	77·9	40·4	71·2	137·9	146·9
	three sprays	5·93	11·57	80·0	86·2	33·3	76·5	178·1	151·2
Jonathan	untreated	4·42	5·99	100	100	33·0	54·4	130·4	110·2
	one spray	4·93	5·41	92·3	90·3	36·9	47·5	133·6	113·9
	two sprays	4·08	4·98	108·8	83·1	30·9	44·4	132·0	112·1
	three sprays	4·81	5·59	108·8	93·3	39·0	47·9	123·3	116·7

Each plot contained four trees of the newly bred Müncheberg varieties Erwin Baur and Musikdirektor Riedel; their shoot growth was unaffected by the TUZET treatment. These varieties are not included in Table 2 because so few specimens were tested.

The results recorded for yield, number of fruits and average fruit weight are listed in Table 3. James Grieve, the first of the tested varieties to mature, produced considerably higher yields, combined with an increased average weight of the individual fruit in 1956 — the first test year — following one and two spray treatments. In the next year, a yield increase of 0·5 kilos per tree and a slight increase in the weight of the individual fruit were registered only on trees which were treated twice.

The values obtained for the Geheimrat Oldenburg variety did not reveal any effects on fruit growth as the result of TUZET treatments in the first year. Frost caused considerable harm to the tree blossom in 1957, resulting in appreciable yield losses, so that this particular year cannot be taken into consideration.

In 1956, the yield for each tree of the Cox's Orange variety — featured by moderately vigorous growth — was in the region of 1·8 kilos in all plots. In 1957, those trees treated twice with TUZET had a yield increase of 0·8 kilo, and in the plots which were sprayed once and three times the yield increase was approximately 0·5 kilo per tree as compared with the untreated plots. The average individual fruit weight gives no indication of any influence having been exerted by the treatment.

The results obtained for Golden Delicious, generally regarded as a sensitive variety, clearly show that the TUZET treatment had negative effects on the yield output in both test years. The yield decrease following a single spray in 1956 was 0·8 kilo, and following two treatments 1·8 kilos per tree. In fact the yield decrease in those plots which were given two treatments amounted in 1957 to 3 kilos per tree as compared with the untreated checks. These yield losses on a variety which already bears fruit on the annual wood, are primarily to be attributed to the reduction in the number of fruit which in 1956 dropped from 53·6 for the untreated plot to 33·3 for the plot sprayed three times with TUZET. Again in 1957, the number of fruit per tree dropped from 94·2 to 76·5 in these plots.

For the Jonathan variety, a yield decrease was recorded in both test years following a single TUZET spray application; several treatments led to a yield increase in the first test year, and a general drop in yield in the second year as compared with the check plots. The figures obtained for the number of fruit produced and the individual fruit weight give no indication of influential effects being exerted in this respect by the crop protection measures.

Table 4: Size grading of Golden Delicious and Jonathan varieties in 1956/1957.

Variety	TUZET treatment	Graded sizes in %							Date of grading
		50/55	55/60	60/65	65/70	70/75	75/80	over 80	
Golden Delicious	untreated	0·3	2·8	11·5	21·9	49·6	13·5	0·4	17. 3. 57
	one spray	0·7	3·5	12·7	22·9	43·2	16·2	0·7	
	two sprays	0·3	5·9	18·4	23·3	41·9	10·3	0·0	
	three sprays	0·3	1·2	9·7	20·2	51·9	14·2	2·6	
Jonathan	untreated	0·9	7·3	33·3	33·7	15·6	6·2	2·3	21.12.56
	one spray	0·0	6·7	34·6	29·5	26·4	2·7	0·0	
	two sprays	0·3	6·7	23·0	36·2	31·6	2·2	0·0	
	three sprays	0·2	6·8	28·5	38·2	20·0	1·3	0·0	

The results obtained for the Erwin Baur and Musikdirektor Riedel varieties are not included in Table 3, either. There were only four specimens in each plot, and it was established that as a result of the TUZET spray applications both varieties showed a yield decrease in the first year followed by a yield increase in the second year as compared with the untreated plots.

In 1956, a grading of sizes was carried out only on the abundantly laden, late varieties Golden Delicious and Jonathan. Table 4 reveals that when the Golden Delicious variety was sorted on March 17, 1957, an increase in the percentage of fruit sizes over 70 mm was registered only for trees which were treated three times.

The Jonathan variety was sorted on December 21, 1956. The results of the size grading showed that the negative influence of the TUZET treatment was negligible.

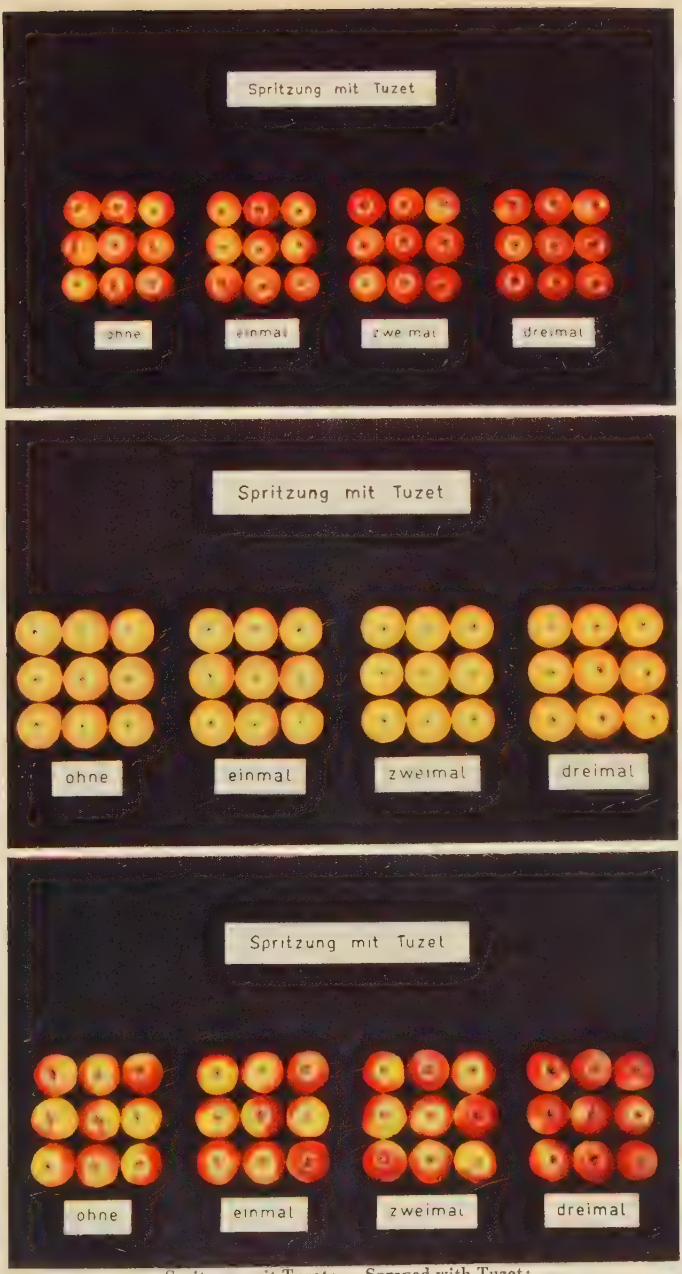
In 1957, all varieties were sorted, with the exception of the Geheimrat Oldenburg variety for which the yields were too small. The figures listed in Table 5 show that for James Grieve, graded on September 6, 1957, only those trees which were treated twice produced approximately 6 % more fruit of over 80 mm in size.

An increase in the percentage of Cox's Orange apples with a size of over 70 mm is established only for those trees sprayed three times with TUZET.

The percentage of big apples of the Golden Delicious variety continued to increase as the number of applied sprays was raised. In addition to the effect of the fungicide, this condition may also be attributed to the decrease in the number of fruit (see Table 3). Thinning out also gives a similar effect.

Table 5: Results of size grading for James Grieve, Cox's Orange, Golden Delicious and Jonathan varieties in 1957/58.

Variety	TUZET treatment	Graded sizes in %						Date of grading
		55/60	60/65	65/70	70/75	75/80	over 80	
James Grieve	untreated	0·6	3·9	24·4	32·1	25·6	13·4	6. 9. 57
	one spray	1·5	3·9	25·9	32·1	25·0	11·5	
	two sprays	0·7	5·1	21·1	30·9	23·0	19·2	
Cox's Orange	untreated	2·8	13·3	32·0	25·6	19·3	11·9	15. 10. 57
	one spray	4·2	13·2	26·6	29·1	16·7	10·3	
	two sprays	8·3	14·9	30·8	23·2	14·3	8·6	
	three sprays	0·0	8·5	18·6	32·1	26·4	14·5	
Golden Delicious	untreated	0·0	17·6	30·5	24·9	23·3	3·7	19. 11. 57
	one spray	0·0	18·6	26·4	26·0	23·4	5·7	
	two sprays	0·0	10·0	25·8	26·5	24·1	6·3	
	three sprays	0·0	6·7	22·8	25·9	32·3	18·3	
Jonathan	untreated	0·2	24·8	46·7	20·1	8·1	0·0	27. 11. 57
	one spray	0·0	24·4	38·3	28·0	9·3	0·0	
	two sprays	0·0	23·7	30·8	23·2	22·3	0·0	
	three sprays	0 0	22·5	47·3	25·6	4·6	0·0	



Spritzung mit Tuzet: = Sprayed with Tuzet;
ohne: = untreated; einmal: = once; zweimal: = twice; dreimal: = three times

Fig. 1—3: Influence of an increasing number of TUZET sprays on the colouring of apples of the Cox's Orange (top), Jonathan (centre) and Golden Dilicious (bottom) varieties in 1956 (calyx at the top)

In 1957, the Jonathan variety was sorted on November 27. It was proved that as the result of one or two TUZET spray applications, the percentage of apples with a size of 75/80 mm increased from 8·1 % for untreated to 9·3 % for a single treatment and 22·3 % for two treatments. Changes in the size of apples on trees which were sprayed tree times, were generally insignificant as compared with the untreated check plots.

Finally, the apples were evaluated with respect to their colouring and keeping quality; the results are listed in Table 6. The dates on which colouring was evaluated are given in brackets. It must be pointed out at this stage that apples produced on trees which are grown on parent stock M IX in hedge formation, are known to acquire a very good colour. Therefore, the object of this study was to establish whether the fruit colour which is good anyway, can be further improved upon by spraying the apples with TUZET. In 1956, the first test year, it was observed that especially the red covering colour was enhanced appreciably on the varieties tested — James Grieve, Geheimrat Oldenburg, Cox's Orange, Golden Delicious and Jonathan — following one or two TUZET treatments. In fact three TUZET spray applications led to a further improvement of colour on Cox's Orange, Golden Delicious and Jonathan. Figs. 1 to 3 give a comparison of 9 apples, size 75/80, selected at random from each plot; they clearly illustrate the effect produced by TUZET especially on the red fruit varieties Cox's Orange and Jonathan. The relatively few apples of the Musikdirektor Riedel variety showed the same reaction as Cox's Orange. No differences were established in the development of the primary colour and the covering colour of Erwin Baur apples.

In the second test year, 1957, the evaluations of colour were carried out separately for apples of sizes 70/75, 75/80 and 80/85 mm, in order to analyze the colour-stimulating effect in relation to fruit size and the natural variation of colour linked with it. As the differences in the primary colour were only slight especially for the red fruit varieties, consideration was given solely to the covering colour for the purpose of the evaluation.

A single TUZET spray application had no effect on James Grieve; two treatments (see Table 6) led to a slight improvement of the covering colour on apples of size 70/75 mm and to a most marked improvement on apples of size 80/85 mm.

The covering colour of Geheimrat Oldenburg apples was enhanced most appreciably following a single spray, and two treatments led to a further improvement.

The effect of one and two spray applications was equal on Cox's Orange. A third treatment produced the greatest improvement of colour that was registered for all varieties, on both size 70/75 and size 80/85 mm. This finding agrees with the results obtained the previous year.

It is known that small apples, especially those of the Golden Delicious variety, have a relatively poor, greenish primary colour, and seldom feature a reddish covering colour. As a result, treatment with TUZET only slightly improved the covering colour on apples of size 70/75 mm. In the case of bigger apples of this variety, the covering colour in particular continued to improve as the number of TUZET spray applications was raised.

The weakest effect was observed in the second test year on the Jonathan variety. Following one and two treatments, the colour was improved slightly only on apples of size 80/85 mm. Three sprays had to be applied in order to enhance the red covering colour on sizes 70/75 mm and 75/80 mm to a degree approaching that attained in the other plots.

Table 6: Colouring and keeping quality of apples of different varieties in 1956 and 1957.

Variety	TUZET treatment	Colouring 1956	Colour evaluation 1957			Keeping quality	Date of evaluation (colour evaluation)
			70/75 mm	75/80 mm	80/85 mm		
James Grieve	untreated	moderate	4	5	6	good	11. 11. 57
	one spray	good	4	5	5	good	(8. 10. 57)
	two sprays	good	5	7	7	good	
Geheimrat Oldenburg	untreated	moderate	4	4	5	good	11. 11. 57
	one spray	good	5	6	6	good	(8. 10. 57)
	two sprays	good	6	6	7	good	
Cox's Orange	untreated	moderate	2	4	4	good	16. 1. 58
	one spray	good	3	4	5	good	(8. 10. 57)
	two sprays	good	3	4	5	good	
	three sprays	very good	5	5	7	very good	
Golden Delicious	untreated	moderate	3	4	5	good	3. 7. 58
	one spray	good	4	5	5	good	(17. 1. 58)
	two sprays	good	4	5	6	very good	
	three sprays	very good	4	6	7	very good	
Jonathan	untreated	moderate	5	6	6	good	3. 7. 58
	one spray	good	5	6	7	good	(17. 1. 58)
	two sprays	good	5	6	7	good	
	three sprays	very good	6	7	7	very good	

The keeping quality of the apples was equally good in both test years, and was not reduced by TUZET treatments on any variety. Cox's Orange, Golden Delicious and Jonathan, stored until the fruit became soft, especially proved to be healthier and to have a more persistent keeping quality than the others even following three TUZET treatments; the losses were also economically tolerable. This applied to Golden Delicious also following two treatments.

In 1957/58, Cox's Orange was stored in perfect quality until early February, with a 15 % loss of apples treated three times with TUZET as compared with a 20 % loss for untreated apples. For Golden Delicious, the losses registered until the middle of March were 2·5 % for apples treated twice and three times, and 5 % for untreated apples. Jonathan, too, only showed a 2·5 % loss for apples treated three times as compared with 6·8 % for untreated apples, at the check in the middle of March. The number of shrivelled apples was smaller in the plots treated with TUZET. At the check carried out on this particular variety on July 3, 1958, there was a 20 % storage loss for apples from the untreated plot and only 9 % of the fruit had not shrivelled, whereas for apples which had been treated three times there was a loss of 12·5 %, and 45 % of the fruit was firm. One and two treatments with TUZET had less favourable effects.

Regarding the russet colouring of the apples, especially for Golden Delicious, no differences were observed at the evaluations in either test year.

Further studies must be carried out to establish the causes of the negative effect of TUZET on shoot growth, yield and number of fruit in the case of Golden Delicious, and to ascertain the influence of this fungicide on the development and vital functions of the leaf. Furthermore, consideration must be given to probable relations between the spraying of arsenic and heavy metals and the stimulation of colour on the fruits.

Summary

Apple trees grown on parent stock M IX were treated over a period of two years with the fungicide TUZET at the plantation of the Institute for Fruit Growing, Bonn. Trees of the James Grieve, Geheimrat Oldenburg, Cox's Orange, Golden Delicious and Jonathan varieties were planted in 1953, and cultivated in hedge formation with horizontal lateral shoots. The trees were in a very good state of health. The TUZET spray applications (one, two and three treatments) were carried out according to the recommendations of the manufacturers *, starting when the apples had grown to the size of a walnut and continued, at decreasing dosages, until four weeks before picking at the latest.

None of the tested varieties showed a significant decrease of shoot growth due to the influence of the TUZET treatment, in either of the test years. Repeated marked reductions in the yields and numbers of apples were observed for the Golden Delicious variety. The reaction of the other varieties to the influence of the TUZET sprays took the form of insignificant deviations of yield increases which were not confirmed in the 2nd. test year, and increases in the number of apples.

The percentage of big apples in the size grading results was raised additively for James Grieve and Cox's Orange by applying three sprays, and for Golden Delicious and Jonathan with increasing frequency of the spray applications. In the first test year, on the other hand, a negative influence was observed for the Jonathan variety.

* cf page 102.

The colour of the apples was improved on all varieties, and in particular the covering colour was enhanced. In order to improve the colour most appreciably, two sprays proved to be necessary for James Grieve and Geheimrat Oldenburg, and three treatments for Cox's Orange, Golden Delicious and Jonathan.

The keeping quality of the apples was not impaired on any of the tested varieties in either of the two test years. The treatments had no perceptible influence on the russet colouring of the apples, especially Golden Delicious.

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